A comparison of whole-body and tissue-specific insulin sensitivity between black west African and white European men with normal glucose tolerance, impaired glucose tolerance and type 2 diabetes

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A comparison of whole-body and tissue-specific insulin sensitivity between black west African and white European men with normal glucose tolerance, impaired glucose tolerance and type 2 diabetes

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Declaration

The data presented in this thesis were collected as part of the South London Diabetes and Ethnicity phenotyping (Soul-DeEP) study. The study was divided into two phases, both funded by Diabetes UK (grant numbers: 12/0004473 and 14/0004967) and awarded to Dr Louise Goff, the principal investigator, and co-investigators Professor Stephanie Amiel, Professor Janet Peacock and Professor Margot Umpleby. I declare that my primary responsibilities and contributions to the overall study were centred around:

- Participant recruitment and characterisation (Soul-DeEP II)
- Day-to-day management of the study (Soul-DeEP II)
- Organising participant metabolic visits (Soul-DeEP II)
- Calculation and preparation of isotope infusions (Soul-DeEP II)
- Plasma glucose readings (Soul-DeEP I & II)
- Sample processing (Soul-DeEP I & II)
- Parts of the laboratory analyses (Soul-DeEP II)
- Data entry, cleansing, analyses and interpretations (Soul-DeEP I & II)
- Academic presentations and publications (Soul-DeEP I & II)

Clinical research fellows; Dr Meera Ladwa, Dr Chinmay Marathe, Dr Cynthia Mohandas, scientific academic Dr Fariba Shojaee-Moradie and qualified research nurses were responsible for executing metabolic assessments and participant safety. Dr Olah Hakim arranged all magnetic resonance imaging scans and quantified fat depots which have been published elsewhere.

Signature:

Name: Rafiat Oluwatoyosi Bello

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Abstract

Introduction: Populations of black ethnicity have a disproportionately high prevalence of type 2 diabetes (T2D) compared to their white counterparts. Adiposity and dysfunctional lipid metabolism have been shown to trigger and exacerbate tissue-specific insulin resistance. Black communities present with pronounced insulin resistance in the presence of lower visceral and ectopic fat. These findings have been demonstrated in participants without T2D and imply there may be an ethnic distinction in the pathophysiology of T2D.

Aim: To use highly sensitive techniques to assess and compare whole-body and tissue-specific insulin sensitivity in black west African (BAM) and white European men (WEM) of normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or type 2 diabetes (T2D), and to assess the association with regional measures of fat and lipolysis.

Methods: Forty-nine BAM (21 NGT, 10 IGT,18 T2D) and 47 WEM (23 NGT, 9 IGT, 15 T2D) underwent a two-step hyperinsulinaemic-euglycaemic clamp with stable glucose and glycerol isotopic tracers to assess insulin sensitivity, and a magnetic resonance imaging scan to assess visceral adipose tissue (VAT) and intrahepatic lipids (IHL).

Results: There were no significant ethnic differences in whole-body or tissue-specific insulin sensitivity between BAM and WEM in any glucose tolerance group ($p>0.05$). VAT and IHL were significantly lower in BAM than WEM in each glucose tolerance group ($p<0.05$). There were no ethnic differences in the associations of peripheral or hepatic insulin sensitivity to glucose homeostasis with VAT or IHL ($p>0.05$). The association between insulin sensitivity to glucose homeostasis with insulin's antilipolytic actions were weaker in BAM. The antilipolytic action of insulin associated with VAT and IHL in WEM but not BAM.

Conclusions: Black men may have resistance to storing of visceral and ectopic fat and lower levels are found in all glucose tolerance groups. This is observed in the presence of similar whole-body and tissue-specific insulin sensitivity, therefore lower visceral and ectopic fat may be a protective mechanism in black men which prevents pronounced insulin resistance. Visceral fat, hepatic fat and a resistance to insulin's antilipolytic action all appear to play a role in insulin resistance to glucose homeostasis in both ethnic groups. The latter association is significantly weaker in black men, and the mechanisms behind this relationship may be dependent on fat accumulation in white men but not black men.

Table of Contents

DECLARATION ..... **II**

ACKNOWLEDGEMENTS ..... **III**

ABSTRACT ..... **IV**

LIST OF PUBLICATIONS ..... **VIII**

LIST OF TABLES ..... **X**

LIST OF FIGURES ..... **X**

LIST OF ABBREVIATIONS ..... **XII**

CHAPTER 1: AN INTRODUCTION TO TYPE 2 DIABETES PATHOPHYSIOLOGY AND ETHNICITY **1**

1.1 Defining type 2 diabetes 2
1.2 The Epidemiology of type 2 diabetes 2
1.3 Risk factors for type 2 diabetes 5
1.3.1 Adiposity as a risk for type 2 diabetes *5*
1.3.2 Ethnicity as a risk factor for type 2 diabetes *6*
1.4 The pathophysiology of type 2 diabetes 13
1.5 Insulin sensitivity 17
1.5.1 Peripheral insulin sensitivity to glucose homeostasis *18*
1.5.2 Hepatic insulin sensitivity to glucose homeostasis *23*
1.5.3 Adipose tissue insulin sensitivity to lipolysis *26*
1.5.4 Other insulin actions and tissues *29*
1.6 Measuring insulin sensitivity 29
1.7 The potential influencers of insulin resistance 34
1.7.1 Visceral adipose tissue *35*
1.7.2 Ectopic fat *40*
1.7.3 Adipose tissue insulin resistance *43*
1.7.4 Other influencers *45*
1.8 Indicators for an ethnic-specific pathophysiology of type 2 diabetes 45
1.9 Insulin sensitivity and black ethnicity 51
1.9.1 Whole-body insulin sensitivity and black ethnicity *52*
1.9.2 Peripheral insulin sensitivity and black ethnicity *54*
1.9.3 Hepatic insulin sensitivity and black ethnicity *55*
1.9.4 Adipose tissue insulin sensitivity and black ethnicity *57*
1.9.5 Insulin sensitivity and black ethnicity summary *58*
1.10 Aims 60
1.10.1 Objectives 60

CHAPTER 2: MATERIALS AND METHODS **61**

2.1 Soul-DeEP study 62
2.2 Participants 64
2.3 Procedures 66
2.3.1 Screening *67*
2.3.2 Hyperinsulinaemic-euglycaemic clamp *67*
2.3.3 Magnetic resonance imaging *73*
2.5 Sample laboratory analyses and materials 74
2.5.1 Automated assays *74*
2.5.2 Isotope enrichment sample procedure *75*
2.6 Calculations 77
2.6.1 Whole-body insulin sensitivity *77*

2.6.2 Tissue-specific insulin sensitivity *79*
2.7 STATISTICAL ANALYSES 85
2.7.1 Power calculation *86*

CHAPTER 3: DESCRIPTION OF PARTICIPANTS 87

3.1 CHAPTER INTRODUCTION 88
3.2 AIM 88
3.3 METHODS 88
3.3.1 Participants and study design *88*
3.3.2 Statistical analyses *89*
3.4 RESULTS 90
3.4.1 Participant recruitment *90*
3.4.2 Characteristics of black west African and white European participants *92*
3.5 DISCUSSION 100

CHAPTER 4: A COMPARISON OF WHOLE-BODY INSULIN SENSITIVITY BETWEEN BLACK AND WHITE MEN 102

4.1 INTRODUCTION 103
4.2 AIM 105
4.3 METHODS 106
4.3.1 Calculations *106*
4.3.2 Statistical analyses *107*
4.4 RESULTS 109
4.4.1 Glucose and insulin during the clamp procedure *109*
4.4.2 Insulin and glucose relationship *113*
4.4.3 Whole-body insulin sensitivity *114*
4.4.4 Whole-body insulin sensitivity adjustments for VAT and IHL *117*
4.5 DISCUSSION 119

CHAPTER 5: A COMPARISON OF PERIPHERAL INSULIN SENSITIVITY BETWEEN BLACK AND WHITE MEN .. 129

5.1 INTRODUCTION 130
5.2 AIM 132
5.3 METHODS 133
5.3.1 Calculations *134*
5.3.2 Statistical analyses *134*
5.4 RESULTS 136
5.4.1 Peripheral glucose disposal *136*
5.4.2 Peripheral insulin sensitivity *137*
5.4.3 Peripheral insulin sensitivity adjustment for VAT and IHL *140*
5.5 DISCUSSION 141

CHAPTER 6: A COMPARISON OF HEPATIC INSULIN SENSITIVITY BETWEEN BLACK AND WHITE MEN 147

6.1 INTRODUCTION 148
6.2 AIM 149
6.3 METHODS 150
6.3.1 Calculations *151*
6.3.2 Statistical analyses *151*
6.4 RESULTS 153
6.4.1 Endogenous glucose production *153*
6.4.2 Hepatic insulin sensitivity *154*
6.4.3 Hepatic insulin sensitivity adjustment for VAT and IHL *159*
6.4.2 Endogenous glucose production during the high dose insulin infusion *160*
6.5 DISCUSSION 161

CHAPTER 7: A COMPARISON OF ADIPOSE TISSUE INSULIN SENSITIVITY BETWEEN BLACK AND WHITE MEN 168

7.1 INTRODUCTION 169
7.2 AIM 170
7.3 METHODS 171
7.3.1 Calculations 172
7.3.2 Statistical analyses 172
7.4 RESULTS 174
7.4.1 Adipose tissue lipolysis 174
7.4.2 Insulin sensitivity to adipose tissue lipolysis 175
7.4.3 Adjusting lipolysis for VAT and SAT 178
7.5 DISCUSSION 179

CHAPTER 8: AN ETHNIC COMPARISON OF THE RELATIONSHIP BETWEEN PERIPHERAL AND HEPATIC INSULIN SENSITIVITY WITH VISCERAL FAT, HEPATIC FAT AND ADIPOSE TISSUE INSULIN SENSITIVITY 186

8.1 INTRODUCTION 187
8.2 AIM 189
8.3 METHODS 190
8.3.1 Calculations 191
8.3.2 Statistical analyses 192
8.4 RESULTS 193
8.4.1 Relationships between peripheral insulin sensitivity and visceral fat 193
8.4.2 Relationships between peripheral insulin sensitivity and hepatic fat 195
8.4.3 Relationships between hepatic insulin sensitivity and fat 197
8.4.4 Relationships adipose tissue insulin sensitivity with peripheral or hepatic insulin sensitivity 199
8.4.5 Relationships between adipose tissue insulin sensitivity and ectopic fat 201
8.5 DISCUSSION 203

CHAPTER 9: DISCUSSION AND CONCLUSIONS 212

9.1 DISCUSSION 213
9.1.1 Strengths and limitations 224
9.1.2 Conclusions 232

REFERENCES 237

APPENDICES (PUBLICATIONS) 262

APPENDIX 1: BLACK AFRICAN MEN WITH EARLY TYPE 2 DIABETES HAVE SIMILAR MUSCLE, LIVER AND ADIPOSE TISSUE INSULIN SENSITIVITY TO WHITE EUROPEAN MEN DESPITE LOWER VISCERAL FAT 262
APPENDIX 2: DIFFERENCES IN THE LINK BETWEEN INSULIN SENSITIVITY AND ECTOPIC FAT IN MEN OF BLACK AFRICAN AND WHITE EUROPEAN ETHNICITY 272

List of publications

Original articles

Bello O, Mohandas C, Shojaee-Moradie F, Jackson N, Hakim O, Alberti KGMM, Peacock JL, Umpleby AM, Amiel SA, Goff LM. Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat. Diabetologia. 2019 May; 62(5):835-844.

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List of tables

Table 1: A summary of the different experimental methods used to measure insulin sensitivity 32
Table 2: Characteristics of black west African and white European men who underwent a hyperinsulinaemic-euglycaemic clamp 95
Table 3: Glucose and insulin concentrations during the different phases of the hyperinsulinaemic-euglycaemic clamp 112
Table 4: Whole-body insulin sensitivity during the hyperinsulinaemic-euglycaemic clamp..... 115
Table 5: Peripheral glucose disposal and peripheral insulin sensitivity before and during the hyperinsulinaemic-euglycaemic clamp 138
Table 6: Endogenous glucose production and hepatic insulin sensitivity before and during the hyperinsulinaemic-euglycaemic clamp 155
Table 7: Adipose tissue lipolysis and adipose tissue insulin sensitivity before and during the hyperinsulinaemic-euglycaemic clamp 176

List of figures

Figure 1: The number of people between ages 20 and 79 diagnosed with diabetes in 2017 and the prediction for 2045. This figure has been reproduced with permission from the International Diabetes Federation atlas eighth edition 2017 (9). 4
Figure 2: The prevalence of doctor-diagnosed type 2 diabetes in the UK for (A) men and (B) women. Data extracted from the Health Survey for England 2004 report on the health of minority ethnic groups (61). 13
Figure 3: The 'egregious eleven' describes eleven distinct physiological changes which encourage the development of type 2 diabetes. American Diabetes Association [The Time Is Right for a New Classification System for Diabetes: Rationale and Implications of the β-Cell–Centric Classification Schema, American Diabetes Association, 2016] (78). Copyright and all rights reserved. Material from this publication has been used with the permission of American Diabetes Association. 17
Figure 4: A schematic diagram for the process of glucose disposal in skeletal muscle with the processes and proteins/enzymes in green stimulated by insulin and the enzyme in red inhibited by insulin. 22
Figure 5: Details for the changes which occur during unhealthy adipose tissue expansion in response to excess caloric intake and reduced physical activity. Dysfunctional lipid storing and adipose tissue remodelling may contribute to the accumulation of visceral and ectopic fat. Created with BioRender.com. 38
Figure 6: Soul-DeEP study design schematic 63
Figure 7: A schematic of the hyperinsulinaemic-euglycaemic clamp protocol and sampling 71
Figure 8: A graphical representation of the plasma glucose (blue line) and dextrose infusion rate (orange line) during a single hyperinsulinaemic-euglycaemic clamp procedure 72
Figure 9: Consort diagram of participant recruitment (Soul-DeEP II) 91
Figure 10: Glucose and insulin concentrations during the clamp in black west African (BAM) and white European men (WEM) with A) normal glucose tolerance B) impaired glucose tolerance and C) type 2 diabetes. Data expressed as mean (SD) per time point. 111
Figure 11: The relationship between glucose and insulin in men with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2D). 113
Figure 12: Whole-body insulin sensitivity in black west African (BAM) and white European men (WEM) by glucose tolerance. 116
Figure 13: Peripheral insulin sensitivity in black west African (BAM) and white European men (WEM) by glucose tolerance. 139
Figure 14: Hepatic insulin sensitivity in black west African (BAM) and white European men (WEM) by glucose tolerance. 158
Figure 15: The percentage of participants who achieved over 90% suppression of endogenous glucose production during the high dose insulin infusion of the clamp split by glucose tolerance and ethnicity. 160
Figure 16: Adipose tissue insulin sensitivity in black west African (BAM) and white European men (WEM) by glucose tolerance. 177

Figure 17: The relationship between peripheral insulin sensitivity with visceral adipose tissue (VAT). 194
Figure 18: The relationship between peripheral insulin sensitivity with intrahepatic lipids (IHL). 196
Figure 19: The relationship between hepatic insulin sensitivity with visceral adipose tissue (VAT) and intrahepatic lipids (IHL)..... 198
Figure 20: The relationship between adipose tissue insulin sensitivity with peripheral and hepatic insulin sensitivity. 200
Figure 21: The relationship between adipose tissue insulin sensitivity with visceral adipose tissue (VAT) and intrahepatic lipid (IHL). 202

List of abbreviations

ANOVA	Analysis of variance
BAM	Black west African men
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
CI	Confidence interval
FFM	Fat-free mass
HDL	High-density lipoproteins
HIRI	Hepatic insulin resistance index
HISI	Hepatic insulin sensitivity index
HOMA	Homeostatic model assessment
IDF	International diabetes federation
IGT	Impaired glucose tolerance
IHL	Intrahepatic lipids
IQR	Interquartile range
IRAS	The Insulin Resistance Atherosclerosis Study
IVGTT	Intravenous glucose tolerance test
LDL	Low-density lipoproteins
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
PISI	Peripheral insulin sensitivity index
Ra	Rate of appearance
Rd	Rate of disappearance
Soul-DeEP	South London Diabetes and Ethnicity phenotyping study
SAT	Subcutaneous adipose tissue
T2D	Type 2 diabetes
TCA	Tricarboxylic acid
TTR	Tracer-to-tracee
UNSD	United nations statistic division
VAT	Visceral adipose tissue
VLDL	Very-low-density lipoproteins
WEM	White European men
WHO	World health organisation

Chapter 1: An introduction to type 2 diabetes pathophysiology and ethnicity

1.1 Defining type 2 diabetes

Type 2 diabetes (T2D) is a chronic, multifactorial, endocrine condition, characterised by dysfunctional macronutrient metabolism (1-4). This dysfunction stems from impaired insulin secretion and sensitivity, leading to persistent hyperglycaemia. The extent of the hyperglycaemia is used to define glucose intolerance and is the cornerstone for the diagnosis of T2D. Glycaemia occurs along a continuum (3); however, various governing bodies have determined cut points for the diagnosis of T2D and intermediate hyperglycaemia ('prediabetes') (5, 6). The consequence of hyperglycaemia is damage to the vasculature which can be subdivided into macrovascular and microvascular (7, 8). The macrovascular complications result from damage to the large vessels through the process of atherosclerosis, leading to peripheral artery disease, coronary artery disease and stroke. Damage to the smaller vessels drives the microvascular complications which include diabetic retinopathy, nephropathy and neuropathy. T2D is a progressive disorder and the risk of these complications increases with the duration and severity of hyperglycaemia. Overall, the complications arising from diabetes increase the risk of mortality and reduce the quality of life (7, 8).

1.2 The Epidemiology of type 2 diabetes

There are multiple forms of diabetes however, T2D accounts for more than 90% of all cases and is therefore, the most prevalent (1, 4). The International Diabetes Federation (IDF) estimated that there were 425 million cases of diabetes across the globe in 2017 (8.8% of all adults), a figure due to rise by almost 50% by 2045 as shown in figure 1 (9). The IDF summary statistics also show that diabetes affects high, middle and low income countries,

with a high expected prevalence rate in continents undergoing westernisation and rapid economic growth. Within the UK, data from the Quality and Outcomes Framework, which is contributed to by almost all general practitioners, determined that over 3.3 million people had been diagnosed with diabetes in 2014 which comprised 5% of the total population (10, 11). Whilst the prevalence of diabetes in the UK was lower in comparison to the mean global prevalence, diabetes and its complications have been estimated to account for 10% of the NHS budget, which highlights its importance as a healthcare priority (12).

1.2 The Epidemiology of type 2 diabetes

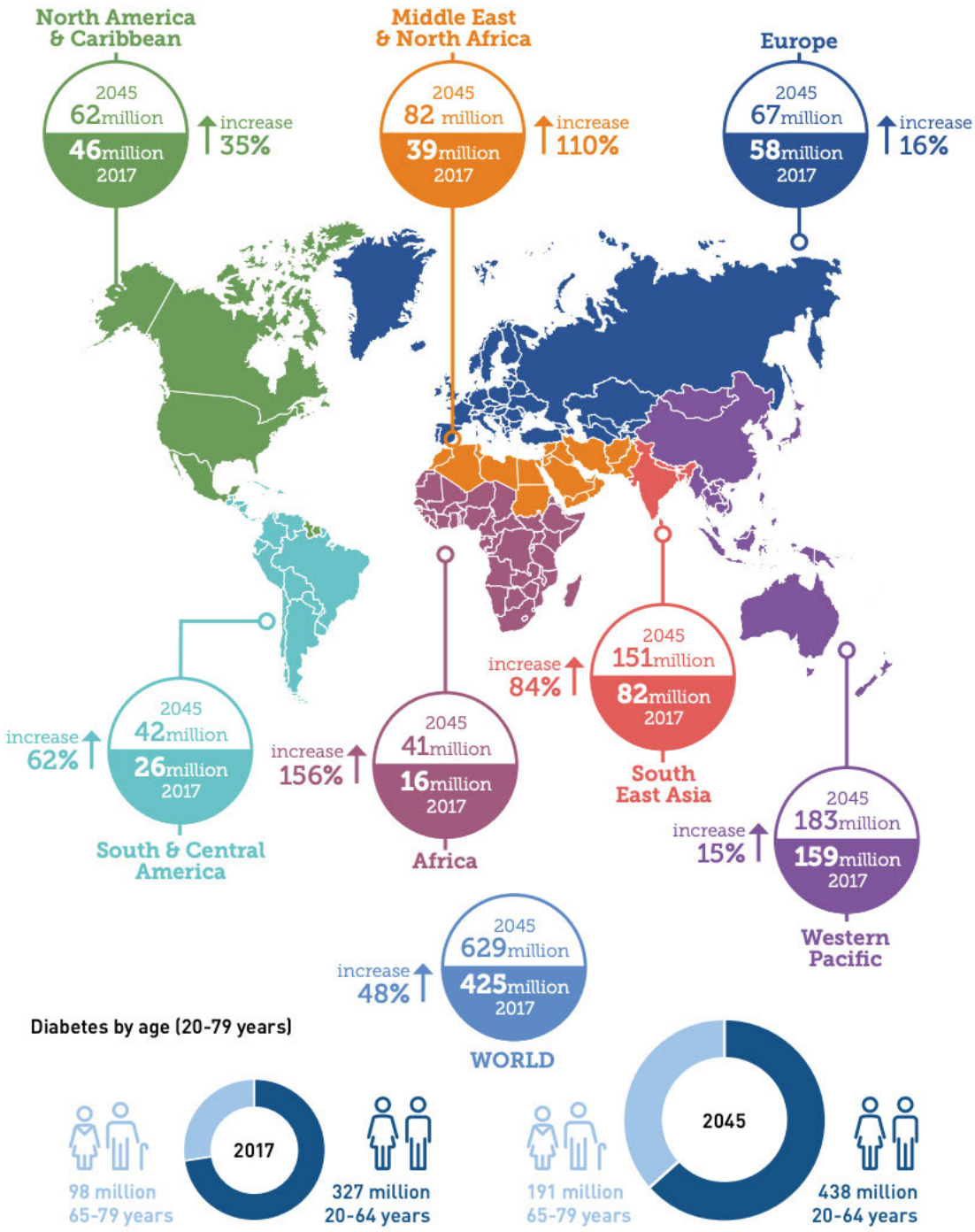


Figure 1: *The number of people between ages 20 and 79 diagnosed with diabetes in 2017 and the prediction for 2045. This figure has been reproduced with permission from the International Diabetes Federation atlas eighth edition 2017 (9).*

1.3 Risk factors for type 2 diabetes

There are numerous factors which show evidence for increasing the risk of the T2D. They can be either genetic factors, environmental factors or both (1, 13, 14). Risk factors include, but are not limited to: a physically inactive and sedentary lifestyle (15, 16); increasing age (4, 17, 18), a positive family history of T2D (19), other medical conditions such as intermediate hyperglycaemia ('prediabetes') or gestational diabetes (3, 13), being overweight or obese (16, 20, 21) and being from an ethnic "minority" background (notably south Asian, Hispanic and black) (1, 22). The latter two risk factors listed have been assessed as part of this thesis and will be the focus going forward.

1.3.1 Adiposity as a risk for type 2 diabetes

Being overweight or obese, as assessed by BMI, is one of the most commonly recognised risk factors for T2D (16, 20, 21, 23). The increased risk with increasing BMI appears to occur independently of other metabolic dysfunctions such as fatty liver or insulin resistance, which highlights the importance of general adiposity (24). However, obesity and general adiposity have not consistently been shown to increase the risk of T2D; the distribution of adiposity has been identified as a more significant risk factor (25, 26). Central adiposity, assessed using the waist circumference, has been shown to be a stronger predictor of T2D than BMI alone (27). Individuals with a high waist circumference but a normal BMI are at greater risk of T2D than those with normal waist circumference and high BMI, this highlights the importance of fat distribution (28).

1.3.2 Ethnicity as a risk factor for type 2 diabetes

In continents across the globe including North America (29, 30) and Europe (31, 32), the prevalence of T2D is consistently reported to be higher in ethnic minority groups compared to white populations. Data from Pima Indians, a group of native Americans , has shown that the incidence of T2D is 19 times higher than the white Americans after adjusting for age and sex (33). In addition, the risk of diabetes being the underlying the cause of mortality is up to 3.5 times higher in comparison to white Americans (34). Longitudinal studies in Pima Indians assessing obesity and T2D physiology have propelled our knowledge of the field and highlighted the impact of insulin resistance (33, 35). Another population which has been shown to suffer from disproportionately high rates of T2D compared to white populations are south Asians. Individuals of south Asian decent include those who live or have roots in India, Sri Lanka, Bangladesh, Pakistan and Nepal. There is a degree of heterogeneity between the T2D prevalence in each south Asian country. India alone, was estimated to have the highest number of individuals suffering from T2D in 2010 at 50.8 million people (36), an estimated prevalence of 16.8% of the population (37). The high prevalence of T2D in south Asia is also observed in migrant populations living outside of south Asia. It has been consistently reported that the risk of diabetes is over 3 times higher in individuals of south Asian decent compared to white European populations (31, 38). Although both South Asians and Pima Indian populations have been demonstrated to exhibit high rates of T2D, a study assessing insulin sensitivity and secretion in over 800 Pima Indians and over 2000 Asian Indians found profound differences. This may suggest that the pathophysiology of T2D is heterogenous among these high risk populations (39).

Finally, populations of black ethnicity have also been consistently identified as a high risk population (31, 40).

1.3.2.1 Heterogeneity within Black populations

Although populations of black ethnicity are described in the literature as Black, Non-Hispanic black, African, African American and Afro-Caribbean; black populations are a highly heterogeneous. Ethnicity is a term used to describe a group of people based on factors such as geographical origins, biological factors and culture (40). Individuals of black ethnicity can descend from Africa however, Africans are a diverse population with different behaviours (including attitudes towards physical activity and diet), risk factors, genetics and disease experiences (41-43), some of which have been discussed below.

In terms of dietary factors, within Africa there is a large degree of heterogeneity in the patterns of food consumption according to region, culture, the economy and environmental factors. Carbohydrates are the common staple however this may in in the form of starchy roots such as yams, fruits such as plantain and grains such a millet or rice. As an example, in south Africa mashed potatoes are a typical component of the diet however this is less commonly consumed in west and east Africa regions (44). As another example, sorghum is a food source grown in very arid locations and is a typical component in central, but not southern, African diets. Interestingly, a study comparing gastric emptying following the ingestion of different carbohydrates in young healthy participants found that gastric emptying from sorghum was significantly slower in comparison to rice potato and pasta. Slower gastric emptying influences post -prandial glycemia in T2D reduces the glucose excursion (44-46). The difference in the common food sources by regions and the data

suggesting that the physiological response to carbohydrates differ depending on the type of carbohydrate, exemplifies the impact that heterogeneity can have. A dietary intake study conducted in the UK further adds to the evidence for differences in dietary intake within the black community. Goff *et al.* conduced an observational assessment of dietary intake in healthy adults who identified as west African or African-Caribbean ethnicity using 24-hour recall. It was found that total energy intake and total sugars were significantly higher in African-Caribbean's compared to west Africans (47).

In terms of genetics, Africa has been highlighted as one of the most genetically diverse continents with a high degree of admixture from non-Africans, particularly in the southern regions. which partially explains the heterogeneity on the continent (43).

In terms of the heterogeneity in disease experience, a recent study comparing over 40,000 non-Hispanic black adults who took part in the U.S. National Health survey, divided participants into African Americans (born in the USA), African immigrants (born in Africa) and African Caribbean (born in the Caribbean). It was found that the prevalence of hypertension, type 2 diabetes and obesity was significantly higher in African Americans in comparison to the other two groups (48), the difference in obesity rates between African Americans and Africans (from rural Ghana) and Caribbean's (from urban Jamaica) has also been reported by a study in 1500 adults (49). As another example, data from population based surveys and the WHO which have assessed the degree of obesity across different regions of Africa have almost consistently reported heterogeneity in the prevalence of obesity and T2D (50, 51). The southern African region (primarily driven by south Africa), and the northern region (primarily driven by Egypt), suffer from a significantly higher burden of obesity in comparison to the remaining African regions (50). Although these examples display a degree

1.3 Risk factors for type 2 diabetes

of heterogeneity in the disease experienced by the black community, detailed studies comparing the pathophysiology of T2D in various black groups are scarce. The studies which have been done suggest glucose, insulin, c-peptide profiles, insulin sensitivity and glucose effectiveness measured during oral and intravenous glucose tolerance tests were no different between 30 Ghanaian immigrants and 68 African Americans with normal glucose tolerance matched for age and BMI (52, 53). A more recent study compared African immigrants residing in Washington and divided participants into those born in western (n=61), central (n=41) and eastern (n=29) Africa (54). The participants were similar in BMI (overweight), diabetes status and blood lipids however the west Africans were significantly older. Each participant underwent an intravenous glucose tolerance test and the results showed no significant difference in insulin sensitivity or secretion (54). This could imply that the differences in the diabetes experience in the different black communities may relate to factors outside of insulin sensitivity or secretion.

1.3.2.2 Black ethnicity as a risk factor for type 2 diabetes

It has been suggested that the disproportionate prevalence of T2D in black communities compared to their white counterparts may partially be attributed to ethnic differences in social and lifestyle factors (such as physical activity and diet) (40).

A systematic review combining studies which compare adults from different ethnic groups found that African Americans consistently participated in less leisure time physical activity compared to white Americans (55). In addition, baseline survey data from the Multi-Ethnic Study of Atherosclerosis (MESA) study; which includes 1503 African Americans and 2425 white Americans, found that African Americans reported less vigorous physical activity

in comparison to white Americans and were more sedentary during leisure hours (15). The importance of this ethnic difference in physical activity is highlighted by the evidence which indicates that the incidence of T2D is higher when physical activity is lower, and the risk of T2D is lower with more moderate to vigorous physical activity (15, 56, 57). Interestingly, this trend appears to be driven more so by the white American population (15) , there may be an ethnic difference in the relationship between vigorous or leisurely physical activity and the incidence of T2D between white and black communities.

Studies which have compared dietary intake in black and white communities have inconsistent results. A study based in the UK using a 4 day food diary in 37 African Caribbean's and 416 white Europeans found that the proportion of total energy intake derived from carbohydrates and starch was significantly higher in African Caribbean's compared to white Europeans (58). This may suggest the African Caribbean diets are of a higher glycaemic load and more likely to be diabetogenic. However, a large US study using data from the 1999–2000 National Health and Nutrition Examination Survey including 7 million African Americans and 41 million Non-Hispanic white Americans found no ethnic difference in carbohydrates but went on to find that adjusting for various macronutrients did not account for the ethnic difference in markers of T2D pathogenesis (namely insulin sensitivity) (59). In agreement, another study in 95 African American and non-Hispanic white children found that adjusting insulin sensitivity for macronutrient intake, measured using 24-hour recall, did not affect the ethnic difference in insulin sensitivity (60). These data could imply that the elevated risk of T2D in black communities is not solely in response to differences in dietary intake.

1.3 Risk factors for type 2 diabetes

1.3.2.3 Black ethnicity as a risk factor for T2D: the perspective in the UK

Within the UK, data from the Health Survey for England in 2004 showed that, compared to white Europeans, the prevalence of T2D was 2-3 times higher in black African or Caribbean men (figure 2A) and women (figure 2B) (61). This disproportionate prevalence has also been reflected in a large-scale epidemiological study by Tillin and colleagues (62). They predicted that by age 80 years, approximately 40 to 50% of black African Caribbeans will have T2D; this is double the predicted prevalence in the white population (62).

Not only is the prevalence higher in black communities, but the relationship with other risk factors appears to be ethnically distinct. Obesity is a risk factor for T2D, which has been discussed in section 1.3.1. However, it is persistently reported that T2D presents itself at a lower BMI in black communities (63-65). Although both obesity and being of black ethnicity constitute as risk factors for T2D, it has become apparent that the association between obesity and T2D may differ between different ethnic groups (66). Data from the UK biobank which includes over 7,000 black participants and 147,000 white participants, has shown that the associations between measures of adiposity (BMI, waist circumference, body fat percentage and hip-to-waist ratio) were significantly steeper in black adults compared to white adults. This relationship remained significantly stronger in black compared to white adults even after adjusting for age, socioeconomic status, physical activity, heart disease, and alcohol consumption. For each unit of BMI (kg/m^2) or waist circumference (cm), the prevalence of diabetes was higher in black compared to white adults (66). In addition to obesity, age is also a risk factor for T2D, which was mentioned in section 1.3. Black populations have been shown to present with T2D 10 years earlier compared to white populations which may imply that the relationship with age is ethnically distinct (63, 67).

Clinical data shows higher rates of T2D complications in black communities, mirroring the increased prevalence and provides evidence for the subsequent healthcare burden (30). Studies from the USA suggest black populations have a higher risk of mortality from stroke, lower limb amputations, end-stage renal disease, diabetic retinopathy and are overall more likely to be hospitalised for diabetes-related complications (30). Studies from the USA also show the response to traditional prevention strategies which target weight loss, conducted outside of the controlled research environment, being significantly less effective in black compared to white communities (68, 69). Whilst studies in the UK assessing the prevention response by ethnicity are scarce, a recent assessment of the UK Diabetes Prevention Programme has reported significantly lower weight loss and less of a reduction in glycated haemoglobin (HbA1c) in individuals of black ethnicity compared to white (70). Therefore, understanding the impact of ethnicity on T2D pathophysiology is a healthcare priority in the UK.

1.3 Risk factors for type 2 diabetes

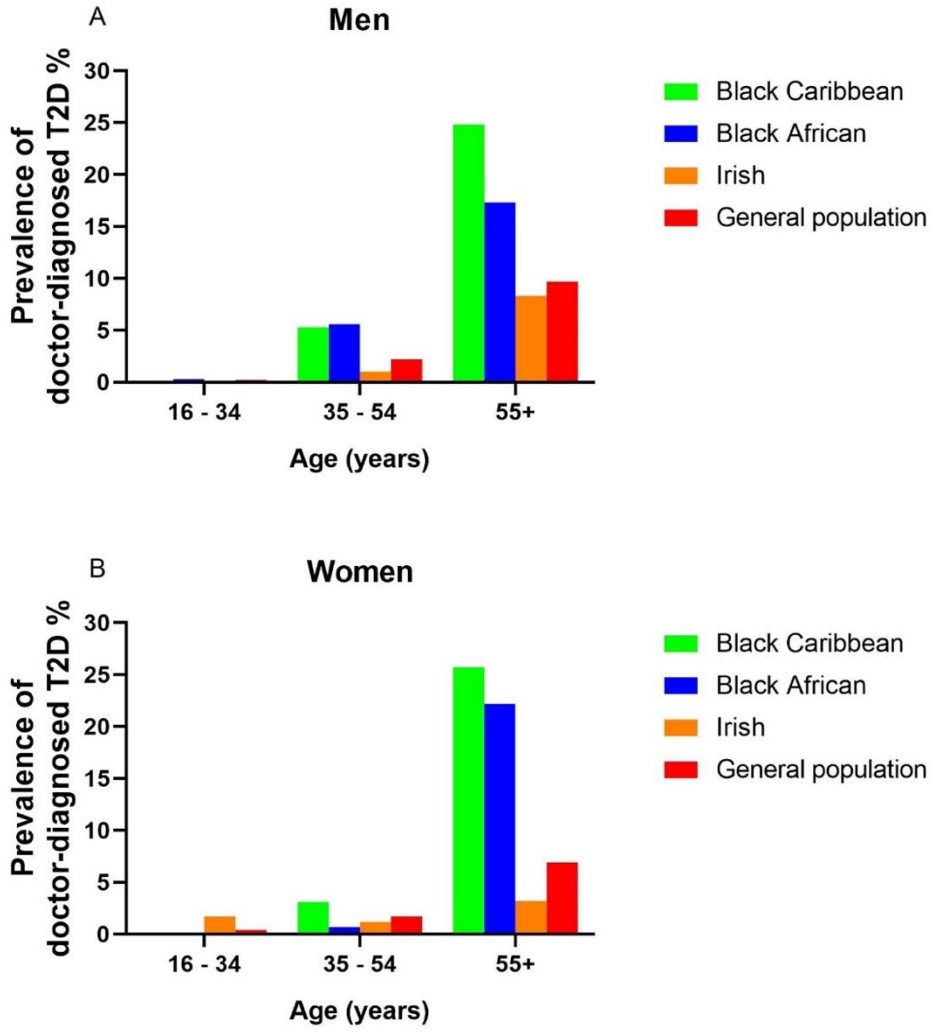


Figure 2: *The prevalence of doctor-diagnosed type 2 diabetes in the UK for (A) men and (B) women. Data extracted from the Health Survey for England 2004 report on the health of minority ethnic groups (61).*

1.4 The pathophysiology of type 2 diabetes

The pathophysiological changes which lead to the development of hyperglycaemia and T2D are complex and involve multiple factors (71, 72). Historically, T2D is described to result from the progressive failure of the pancreatic beta-cell to secrete sufficient insulin, on a backdrop of insulin resistance (1, 72). A resistance to insulin's action, particularly in the muscle and liver, has been shown to occur early in the development of T2D. It is thought to

result from either, or both, genetic susceptibility and a positive energy balance linked to environmental, socioeconomical and behavioural factors. The pancreatic beta-cell is sensitive to changes in blood glucose and over-secretes insulin to compensate for the insulin resistance which prevents frank hyperglycaemia from occurring. The beta-cell compensatory response encompasses adaptive changes. The beta-cell increases proliferation to increase beta-cell mass; although evidence in humans is minimal due to the lack to techniques and cadaver studies show a large range in beta-cell mass of patients with T2D, and upregulates intracellular mechanisms (unfolded protein response and mitochondrial metabolism) to increase beta-cell performance. The prolonged insulin secretory response to the hyperglycaemia is suggested to cause stress to the endoplasmic reticulum, oxidative stress, mitochondrial dysfunction and inflammation. This leads to either beta-cell death (apoptosis) or beta cell differentiation into a new phenotype with altered insulin signalling pathways and insulin production pathways, thought to protect the cell from death (73, 74). During the stage of beta-cell hypersecretion, patients present with hyperinsulinemia which is also thought to lead to a vicious cycle of worsening insulin resistance and the need for more insulin production. Overt T2D develops in individuals who fail to elicit the compensatory insulin secretion and whose beta-cell function declines allowing hyperglycaemia to occur (71, 72, 75, 76). Longitudinal data from the Whitehall study based on over 6000 participants who were followed up for 9.7 years on average and assessed using fasting insulin secretion indices suggest that beta cell function declines exponentially (77).

In 2009, the term ‘ominous octet’ was conceived and summarised the evidence for eight distinct factors which could drive T2D. However, since 2016 the ‘ominous octet’ has been updated to the ‘egregious eleven’ as shown in figure 3 (78). Whilst the traditional

description of the progression to T2D remains at the centre of the literature exploring the pathophysiology, the ‘egregious eleven’ details the complexity of the pathophysiological changes and identifies the pancreatic beta-cell failure as the final common denominator for hyperglycaemia. The beta-cell failure describes the decline in function and/or a loss of the cell mass (apoptosis), leading to a decline in endogenous insulin secretion; also described as dysfunctional beta cell mass. Similar to insulin resistance, the tendency towards beta-cell failure to adapt and elicit a compensatory response may depend on if a genetic susceptibility is present (78). In addition to the final common denominator, beta-cell failure, the other distinct pathological processes which promote the development of T2D are summarised as follows:

- A reduction in the incretin effect, which normally functions to augment insulin secretion, is thought to contribute towards hyperglycemia. In a healthy individuals, an oral glucose load will stimulate the gut to release incretin hormones (glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1) which potentiate glucose induced insulin secretion (79).
- A defect in the pancreatic alpha-cells, which secrete glucagon when glucose concentrations are below the required demand, has also been suggested to promote hyperglycemia. Glucagon releases glucose from glycogen stores in the liver resulting in an increase in circulating glucose and may contribute towards the elevated fasting plasma glucose seen in T2D (71, 80, 81).
- An increase in tissue-specific insulin resistance is often described as a driving factor for the increased demand on the beta-cells to secrete more insulin, which leads to the beta-

cell failure and contributes towards hyperglycemia. Insulin resistance can be divided based on the different sites of insulin action -

  - Peripheral insulin resistance; a decrease in insulin mediated peripheral glucose uptake. The peripheral tissues are primarily composed of skeletal muscle.
  - Hepatic insulin resistance; an increase in endogenous glucose production in the presence of insulin. The liver is the primary source of endogenous glucose production.
  - Adipose tissue insulin resistance to lipolysis; increased lipolysis in the presence of insulin (71).

- Changes to the neuronal activity which regulates energy homeostasis and metabolism such as increasing appetite which can contribute towards hyperglycemia. This may represent a form of insulin resistance because insulin has been shown to reduce appetite (71, 78, 82).
- Alterations in gut microbiota have been associated with changes in insulin sensitivity and products produced by the microbiome fermenting macronutrients have receptors on beta cells which have been shown to augment insulin secretion in vitro (82).
- A low grade chronic systemic inflammatory response by the immune system has been associated with T2D and insulin resistance. Inflammatory cytokines have been shown to induce beta-cell damage, apoptosis and reduced function which may contribute towards hyperglycemia (83).
- Increased gastric emptying and glucose absorption in the small intestine may contribute towards hyperglycemia. This may occur in response to reduced amylin production as a result of beta-cell dysfunction (78).

1.4 The pathophysiology of type 2 diabetes

- Dysfunctional glucose handling in the kidneys is thought to exacerbate hyperglycemia. Mild hyperglycemia upregulates SGLT-2 which is protein required for glucose reabsorption. The consequence of this response is a retention of excess glucose by the kidneys leading to a cycle of progressive hyperglycemia (71, 78).

This thesis is focused on assessing decreased peripheral glucose uptake, increased hepatic glucose production, and increased lipolysis, all of which occur in insulin resistance.

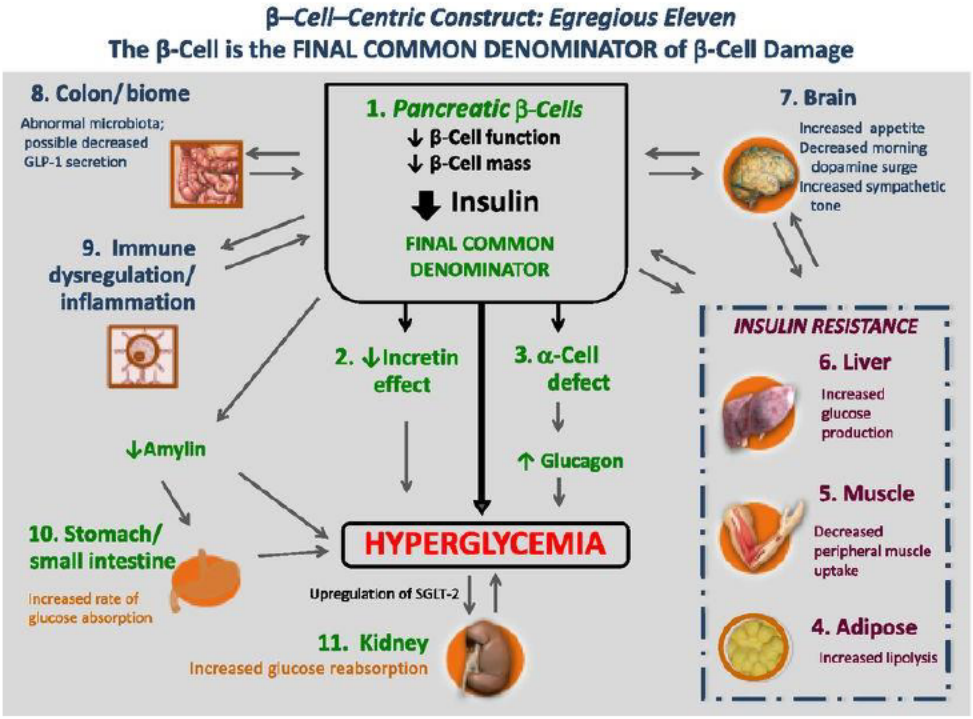


Figure 3: *The 'egregious eleven' describes eleven distinct physiological changes which encourage the development of type 2 diabetes. American Diabetes Association [The Time Is Right for a New Classification System for Diabetes: Rationale and Implications of the β-Cell–Centric Classification Schema, American Diabetes Association, 2016] (78). Copyright and all rights reserved. Material from this publication has been used with the permission of American Diabetes Association.*

1.5 Insulin sensitivity

Insulin sensitivity is defined as the responsiveness of tissues to insulin. The decline of insulin sensitivity, termed insulin resistance, is a precursor for the development of T2D

(84). Insulin sensitivity occurs along a spectrum and whilst a few studies have attempted to determine the cut-off at which insulin sensitivity is pathologically low (insulin resistant) (84, 85), this has yet to be replicated extensively in the literature or clinical practice. Insulin resistance is defined as a metabolic state by which higher than normal insulin concentrations are required to elicit a quantitively normal response; it represents a state of impaired insulin response (86, 87). Insulin is well known for its glucose-lowering actions (88), and the term insulin sensitivity is generally used to describe insulin-mediated glucose disposal and suppression of endogenous glucose production. However, insulin is a peptide hormone secreted from the beta cells of the pancreas into the circulation where it acts on cell surface receptors on multiple cell types and tissues. This pleiotropic nature of insulin suggests that insulin resistance can be tissue-specific (89, 90). Tissue-specific insulin receptor knockout models in rodents have confirmed this tissue-specific nature of insulin sensitivity *in vivo* (91-94). Understanding insulin actions at different sites provide a better understanding of T2D pathophysiology and potential targets for treatment and prevention. The organs and tissues which insulin target that have been identified as dysfunctional in T2D include the peripheral tissues and the liver.

1.5.1 Peripheral insulin sensitivity to glucose homeostasis

When considering the actions of insulin, the peripheral tissues are effectively comprised of skeletal muscle and adipose tissue, both of which have significant involvement in glucose metabolism (95). Glucose is taken up and metabolised in peripheral tissues, referred to as peripheral glucose disposal. Insulin has been well characterised as a regulator of glucose disposal by increasing the transport of glucose into the peripheral tissues and

influencing the intracellular fate of the glucose (oxidative or non-oxidative metabolism). Thus, peripheral glucose disposal increases during the insulin-stimulated state, e.g. after digestion of a meal. An early tracer study provided evidence that adipose tissue glucose uptake accounted for less than 1% of peripheral disposal from intravenous glucose (96, 97). Since then, other studies using positron emission tomography with tracers and the hyperinsulinaemic clamp have shown adipose tissue to account for less than 5% of total glucose disposal (75, 95, 98, 99), although this percentage can be higher in cases of severe obesity (BMI over 43 kg/m^2) due to the higher volume of adipose tissue (100-102). In comparison, studies (mainly in healthy participants) which have quantified the contribution of skeletal muscle glucose uptake to peripheral or whole-body glucose disposal using femoral artery or vein catheterisation or positron emission tomography with tracers, consistently show much higher percentages which range from 70 to 90% (76, 86, 90, 95, 97, 103-106). These data indicate that peripheral glucose disposal primarily reflects disposal into the skeletal muscle.

1.5.1.1 Peripheral insulin sensitivity, a focus on the cellular process

Insulin promotes peripheral glucose disposal by affecting multiple cellular processes which are summarised schematically in figure 4. To begin peripheral glucose disposal, insulin increases the rate of glucose transport across the cell membranes into peripheral tissue cells; it does so by binding to and activating the insulin cell surface tyrosine kinase receptor. When active, the insulin receptor substrates become phosphorylated and a downstream intracellular signalling cascade of substrate phosphorylation dephosphorylation reactions occur. This cascade results in the translocation of intracellular glucose transporters (the

GLUT 4 isoform) to the cell membrane (95). Glucose can then enter the cell through the GLUT 4 transporters. Glucose entry into peripheral tissue cells occurs primarily through GLUT 4 transporters and this process is highly regulated by insulin (107). Once glucose enters the cell, it is rapidly phosphorylated by a hexokinase enzyme (activated by insulin) which traps the glucose in the cell for utilisation. The phosphorylated glucose, glucose 6–phosphate, is either stored as glycogen or oxidised for energy by being converted to pyruvate then acetyl-coA which can enter the TCA/Krebs cycle in the mitochondria to generate energy (95).

The storage of glucose as glycogen is further regulated by insulin. Insulin activates the glycogen synthase enzyme (which drives glycogenesis) and is highly sensitive at inhibiting the action of glycogen phosphorylase, which breaks down glycogen. The combined effect is a net increase in glycogen, which is later utilised when blood glucose levels decline (107). Glucose is stored in the adipose tissue as glycogen but to a lesser extent than in muscle (100, 108), for which the function is less clear. Glucose which is not stored directly as glycogen is oxidised to release energy and other metabolic by-products. This breakdown begins with glycolysis which involves a series of enzymatic reactions also regulated by insulin (109). In particular, insulin activates the enzyme 6-phosphofructokinase which is part of the glycolytic chain of enzyme reactions (107, 110, 111). The end-stage product of glycolysis is pyruvate, which when oxygen is present (oxidative metabolism), is transferred to the mitochondria and converted to Acetyl-coA and joins the Krebs/TCA cycle which releases energy, CO_2 and H_2O. Glucose also undergoes glycolysis in the adipose tissue, and this forms intermediate by-products which increase *de novo* lipogenesis (the formation of fatty acids and or glycerol which are subsequently esterified into triglycerides)

1.5 Insulin sensitivity

in the adipose tissue (100, 109, 112, 113). However, the magnitude of fatty acids formed from *de novo* lipogenesis is not as high as the fatty acid uptake which is discussed in section 1.5.3 (100).

It is thought that when oxygen is limited, e.g. during anaerobic physical activity, the pyruvate which represents the end-stage of glycolysis, is converted to other glucogenic intermediates such as lactate or alanine. This process releases less energy than oxidative metabolism. The lactate is released from the muscle and taken up by the liver for conversion to glycogen, lactate is a precursor molecule for this process (109). The molecular process for the conversion of glucose to lactate to glycogen in the liver is termed the Cori cycle (107). Overall, the direct conversion of glucose to glycogen and the indirect process of lactate to glycogen (glycogenesis) are described together as non-oxidative glucose metabolism (95). Data from a single tracer study in 11 normal glucose tolerant (NGT) participants free from a family history of T2D quantified glucose disposal following a meal (6 kcal/kg) composed of 50% carbohydrate, 30% fat and 20% protein. Woerle *et al.* found that 33% of glucose entering the cell undergoes direct storage as glycogen; the remaining 67% undergoes glycolysis. This can either be oxidative (44%) or non-oxidative (23%) forming glucogenic intermediates such as lactate. 12% of these nonoxidative outputs are stored in the liver as glycogen and 11% are used by the liver in glucogenesis which reforms glucose (114).What is not addressed by Woerle *et al.* is that the fate of glucose differs when glycogen stores are at capacity or depleted and that metabolic demand for the individual varies. Therefore, it is likely that there is a range in the percentage of glucose which is stored or undergoes glycolysis. Factors which may influence the glycogen storage status include prolonged fasting, moderate-to-high intensity exercise or increased intake of fructose (115). In addition

to metabolic state, the method used to measure glucose storage may also play a role in differences in the reported fate of glucose. Studies based on euglycaemic insulin clamp, which use intravenous rather than oral glucose, have provided evidence that 60-90% of glucose is stored as glycogen and the remaining is oxidised for energy via glycolysis (95, 116, 117).

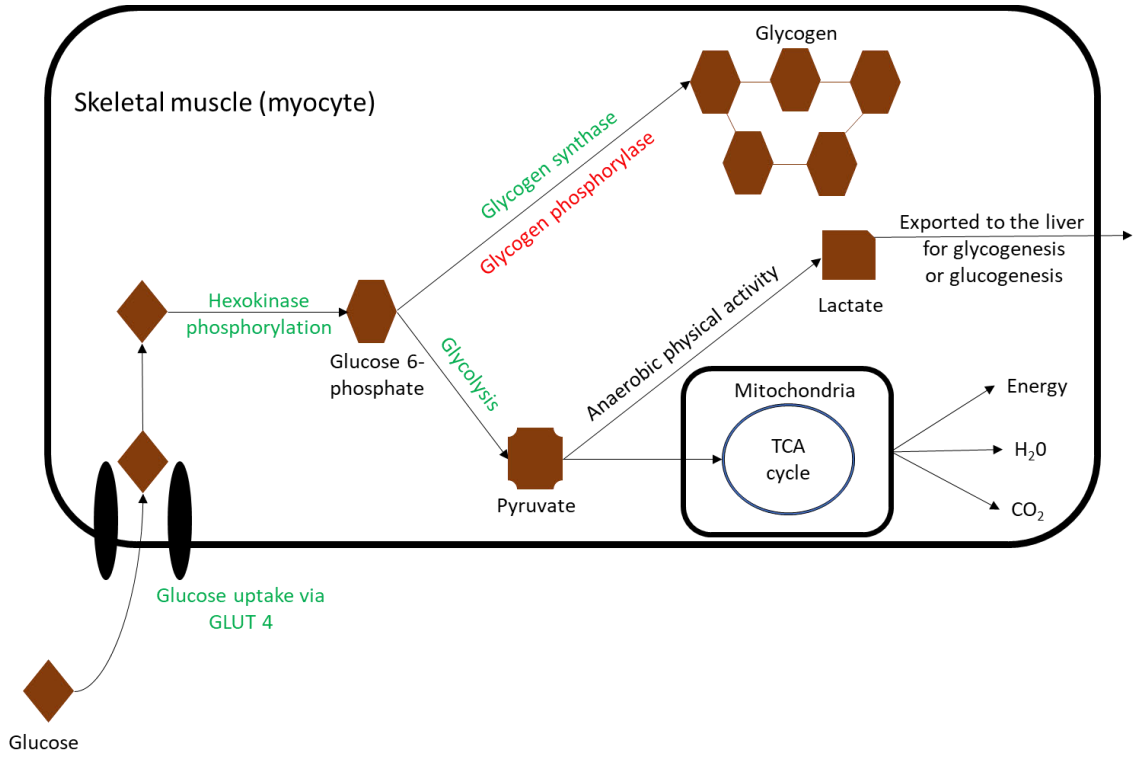


Figure 4: *A schematic diagram for the process of glucose disposal in skeletal muscle with the processes and proteins/enzymes in green stimulated by insulin and the enzyme in red inhibited by insulin.*

1.5.1.2 Peripheral insulin sensitivity changes with impaired glucose tolerance

In impaired glucose tolerance, peripheral insulin sensitivity for glucose metabolism becomes moderately to severely defective. Data from longitudinal studies in Pima Indians have been used to assess the progression of T2D (95). 24 NGT participants with an average age of 27, an average BMI of 37.6 kg m^2/kg at their initial assessment, progressed to impaired glucose tolerance (IGT) within the 2 year follow up. Participants underwent a

Hyperinsulinaemic-euglycemic clamps and oral glucose tolerance tests. The data show an almost consistent fall in peripheral insulin sensitivity (insulin mediated glucose disposal) and a rise in hyperinsulinemia when comparing the initial and follow up assessments (118). In addition to longitudinal studies, comparing NGT participants with a first-degree relative suffering from T2D is thought to provide an insight into the disease progression. This is particularly apparent in Mexican-Americans who have up to an 80% increased risk of T2D when there is a positive family history of T2D (95). Multiple studies in Mexican-American adults (119, 120) and white Europeans (119, 121, 122) have provided evidence for greater insulin resistance in normal glucose tolerant individuals with positive family history of T2D compared to those with a negative family history of T2D. The pronounced insulin resistance found in individuals with a family history and the longitudinal data in particular, suggest that that peripheral insulin resistance may be implicated in the development of T2D .Peripheral glucose disposal in response to insulin has been shown to decline by over 45% in lean and obese subjects with T2D (75, 86, 90, 97) and is approximately 30% lower in impaired glucose tolerance (IGT) compared to normal glucose tolerance (NGT) (123). Studies have shown that the peripheral insulin resistance is mainly a reflection of impaired glycogen synthesis (non-oxidative glucose metabolism) rather than oxidative glucose metabolism (95, 106, 124-126) although earlier work shows impairments in both oxidative and nonoxidative glucose metabolism in T2D compared to NGT (127).

1.5.2 Hepatic insulin sensitivity to glucose homeostasis

The liver orchestrates a multitude of metabolic reactions which contribute to glucose homeostasis. The processes which are well characterised include hepatic gluconeogenesis,

glycolysis, glycogen synthesis (glycogenesis) and glycogenolysis, which collectively contribute to net hepatic glucose production. Insulin, in combination with other hormones and substrates, influences these processes and is well characterised to mediate suppression of hepatic glucose production (128). Skeletal muscle is primarily responsive to insulin during the stimulated state where it takes up exogenous glucose and any residual hepatic glucose production from the liver. The liver is also sensitive to insulin at lower concentrations which include those of the basal/fasted state. During such, the primary source of glucose (fuel) to glucose-requiring organs, e.g. neural tissues, is endogenous glucose production. This is true across glucose tolerance groups. The organs which contribute to endogenous glucose production are primarily the liver (95%) and the extrahepatic glucogenic organs (mainly from the kidneys; 5%)- therefore, hepatic glucose production is the main component of endogenous glucose production, which highlights the importance of the liver in endogenous glucose regulation (128-130).

1.5.2.1 Hepatic insulin sensitivity, a focus on the cellular process

Unlike the muscle, glucose entering the liver occurs primarily independently of insulin through GLUT 2 glucose transporters; therefore, insulin does not increase hepatic glucose uptake (131). Glucose which enters the liver cell (hepatocyte) is phosphorylated by glucokinases, rather than hexokinases found in the muscle, which prevents glucose from being exported by the hepatocyte (132). The activation of glucokinases is facilitated by insulin; therefore, insulin plays a role in retaining glucose which enters the hepatocyte. The glucose is subsequently used as a substrate for glycogen synthesis and for hepatic *de novo* lipogenesis (the production of fatty acids which can be esterified into triglycerides and

packaged into very-low-density lipoproteins (VLDLs) for transport in the blood) when glycogen stores have reached capacity (113, 133, 134). Similar to muscle, glycogen synthesis and glycolysis also occur in hepatocytes and are regulated by insulin as described in section 15.1.1.

In addition to taking up glucose, the liver is the primary source of glucose production during the fasted state. As discussed previously, insulin is well characterised as a suppressor of hepatic glucose production, particularly during feeding when glucose and insulin concentrations are high. Insulin suppresses hepatic glucose production by inhibiting gluconeogenesis and increasing glycogen synthesis during the insulin-stimulated state. There is evidence to support that insulin receptor signalling increases glycogen synthesis by activating the glycogen synthase. There is also evidence for insulin-stimulated transcription of the FOXO1 gene which reduces gluconeogenesis; however, this effect is much slower. Insulin's combined impact of reducing gluconeogenesis and increasing glycogen synthesis is reduced hepatic glucose production. Insulin also has indirect actions on the hepatocyte which further reduce hepatic glucose production through reducing gluconeogenesis. Insulin inhibits lipolysis in the adipose tissue which reduces the availability of glycerol and fatty acids to the liver. Both glycerol and fatty acids can be taken up by hepatocytes and used as a substrate for gluconeogenesis. Insulin reduces this indirectly by inhibiting adipose tissue lipolysis, which releases glycerol and fatty acids, therefore reduces the substrates for gluconeogenesis (107, 128, 135, 136).

1.5.2.2 Hepatic insulin sensitivity changes with impaired glucose tolerance

Studies comparing NGT with IGT show no difference in hepatic metabolism (endogenous glucose production) suggesting dysfunctional hepatic metabolism may not contribute to IGT (123). However, individuals diagnosed with frank hyperglycaemia (T2D) have consistently shown reduced insulin-stimulated suppression of hepatic glucose production during physiological and supraphysiological conditions. The basal release of hepatic glucose is also significantly higher in T2D which contributes to fasting hyperglycaemia (128). The increased hepatic glucose production occurs in the presence of hyperinsulinaemia and is termed hepatic insulin resistance. Reduced suppression of hepatic glucose production characterises hepatic insulin resistance which is often found in T2D and contributes to hyperglycaemia in both the postabsorptive (fasting) and postprandial (feeding) state. Studies comparing NGT and T2D metabolism have shown impairments in the hepatic insulin receptor signalling in hepatocytes and impairments in the indirect insulin action (increased lipolysis end products), both of which result in increased gluconeogenesis. Glycogenolysis has not been shown to change in T2D (128, 132); however, the dual effect of increased gluconeogenesis and no change in glycogenolysis is an increase in hepatic glucose production which contributes to hyperglycaemia.

1.5.3 Adipose tissue insulin sensitivity to lipolysis

Adipose tissue was historically thought to be a metabolically inactive tissue which stored lipids as triglycerides. However, it is now understood to have several metabolic functions which affect glucose and lipid homeostasis (107, 137). As discussed in section 1.5.1, adipose tissue forms part of the peripheral tissues which are subject to insulin-

stimulated glucose disposal. The adipose tissue contribution to peripheral glucose uptake has been shown to be marginal in comparison to the muscle (100). An assessment of adipose tissue glucose uptake in obese and lean participants has shown that in the presence of lower whole-body insulin sensitivity in obese participants, adipose tissue glucose uptake was lower in obese compared to lean participants when expressed per unit of fat mass. However, when multiplied by fat mass to reflect total adipose tissue glucose uptake, there was no difference in adipose tissue uptake between lean and obese participants due to the higher fat mass in obesity. For both lean and obese participants, adipose tissue glucose uptake accounted for less than 5% of total glucose uptake and it was suggested that resistance to adipose tissue glucose uptake may not be the culprit for whole-body insulin resistance (98).

This section focuses on insulin's effects on lipid metabolism in the adipose tissue (the adipocytes). Insulin is known for its actions in reducing circulating fatty acids. It does so in part, by inhibiting lipolysis in the adipose tissue. Lipolysis is defined as the hydrolysis (break down) of triglycerides into glycerol and fatty acids (138). Insulin is a potent antilipolytic hormone, this action is sensitive at lower concentrations of insulin than insulin-mediated glucose disposal in the muscle. Insulin inhibits adipose tissue lipolysis by binding to and phosphorylating the insulin tyrosine kinase receptor which triggers a downstream signalling cascade. This cascade ends with reduced phosphorylation and thus inactivation of hormone-sensitive lipase, an enzyme which cleaves triglycerides as part of the lipolysis process (72, 100, 107, 139-142).

In addition to insulin-regulated lipolysis in adipose tissue, lipolysis can also occur extracellularly in the circulation (138); although the magnitude of this is minimal in comparison to intracellular lipolysis (143-145). Triglycerides are hydrophobic molecules,

and the circulation is an aqueous environment. Therefore, the primary source of circulating triglycerides is within triglyceride-rich lipoprotein complexes (e.g. chylomicrons or VLDLs) (146). Lipoprotein lipases are a group of enzymes which hydrolyse triglycerides within the circulating lipoproteins and therefore facilitate triglyceride clearance (135). This action releases fatty acids and glycerol which are taken up by adipose tissue or muscle by passive diffusion or through fatty acid transporters. They are either used to produce energy via fatty acid oxidation or are re-esterifed into triglycerides for storage; depending on the cellular requirement (145). This process particularly occurs during the fed, insulin-stimulated state. Studies have shown that insulin increases the expression and activity of lipoprotein lipase (107, 146-148) and increases the translocation of fatty acid transporters (e.g. CD36 or FATP1) which facilitate fatty acid entry into adipose tissue primarily for storage (100, 107, 137, 147, 149, 150) or muscle (145).

Overall data suggest insulin promotes fat accumulation through increasing storage and mobilisation of triglycerides (as discussed here) and by stimulating *de novo* lipogenesis which occurs when glycogen stores reach capacity during glucose uptake (as discussed in section 1.5.1.1) (100, 113).

1.5.3.1 Adipose tissue insulin sensitivity changes with impaired glucose tolerance

Resistance to the antilipolytic action of insulin in adipose tissue has been implicated in the development of T2D (151-154). This resistance results in excessive fatty acids release in the presence of insulin. A cross-sectional study has shown that adipose tissue insulin resistance to lipolysis is present in obese NGT, IGT and T2D, with insulin resistance getting progressively worse. This provides evidence for the role of adipose tissue insulin resistance

in T2D development. On average, adipose tissue resistance to insulin stimulated fatty acid suppression was 2.5 and 4 times higher in IGT and T2D, respectively, compared to lean NGT (154); although differences in fat mass and age were not accounted for in that study. Similar findings have also been found in adolescents when comparing NGT and IGT in a cross-sectional study (153).

1.5.4 Other insulin actions and tissues

Sections 1.5.1 and 1.5.2 discuss insulin's effect on glucose metabolism in peripheral and hepatic tissues; however, insulin is also able to affect lipid metabolism in peripheral and hepatic tissues. In skeletal muscle, insulin decreases the oxidation of fatty acids which is thought to compete with glucose oxidation as described in the Randle cycle (107, 155). Insulin also has actions which influence protein metabolism in the muscle and liver (107). These aspects of lipid and protein metabolism are beyond the scope for this thesis but should also be noted.

The descriptions in sections 1.5.1 and 1.5.2 have focused on insulin-mediated glucose disposal and metabolism however, glucose disposal also occurs independently of insulin. This is mainly present in neural and some splanchnic (intestine) tissues. The glucose disposal in these tissues is not thought to change in the development of T2D and is also beyond the scope for this thesis (105).

1.6 Measuring insulin sensitivity

Despite the many actions of insulin, it is the insulin-mediated glucose disposal and suppression of endogenous glucose production that is generally referred to as 'insulin

sensitivity' in the context of T2D (89). Andres, DeFronzo and colleagues developed and published the hyperinsulinaemic-euglycaemic clamp (HEC) as a method for assessing insulin sensitivity *in vivo* (156-158). The principles behind this technique are based on the creation of a hyperinsulinaemic environment by infusing insulin peripherally and allowing the concentration to achieve a plateau. In order to maintain euglycaemia during the hyperinsulinaemia, an exogenous glucose infusion is simultaneously applied. The glucose infusion rate required to achieve euglycaemia in the presence of hyperinsulinaemia, is a measure of an individual's sensitivity to insulin. This method assumes that the peripheral insulin infusion suppresses any contribution which endogenous glucose production may have, so that the glucose infusion is the only glucose source. The glucose infusion rate is, thusly, a measure of total glucose disposal or whole-body insulin sensitivity and is expressed as the M value (87). To compare this M value between different individuals, it must be normalised by expressing the infusion rate per unit of metabolic size. There are multiple options such as kg body weight, fat-free mass (FFM) or body surface area (BSA). Interestingly, data from the Pima Indian population has identified that normalising by kg body weight underestimates insulin sensitivity during the obese state because obese individuals have a greater proportion of lower metabolising adipose tissue. FFM is the ideal option due to the insulin sensitive nature of FFM. Less data are available on the correction for BSA as a surrogate assessment of metabolic size (33, 84, 104). Insulin sensitivity can also be expressed as a function of insulin by normalising total glucose disposal per unit of insulin (156).

The pleiotropic nature of insulin suggests assessing the insulin response in each tissue, beyond assessing solely total glucose uptake, is of interest in T2D pathophysiology

(89). By tracing glucose and glycerol fluxes using isotopic tracers before and during a hyperinsulinaemic-euglycaemic clamp, tissue-specific (peripheral, hepatic and adipose) assessments of insulin action can be derived (105, 143, 144, 159-161). There are multiple methods for expressing insulin sensitivity derived from the hyperinsulinaemic-euglycaemic clamp and isotopic tracers. However, there is no consensus for the optimal calculation to express insulin sensitivity from clamp measures. Various investigators have employed percentage change, insulin adjustments and other calculations across the literature (87, 89, 162). The most appropriate calculation is dependent on the research question and the characteristics of the individuals analysed. Peripheral insulin sensitivity is generally assessed during insulin stimulation with the variations being whether the glucose disposal is presented alone, whether glucose disposal is adjusted for ambient insulin concentrations (i.e. using the peripheral insulin sensitivity index (153)), or whether the percentage increase in glucose disposal is presented (163). In comparison, both hepatic and adipose tissue insulin sensitivity are frequently assessed during the basal/postabsorptive state using isotopic tracers without insulin stimulation (164-167) or as the suppression of glucose or glycerol appearance during insulin stimulation (129, 163).

Bergman and colleagues have also developed and published a dynamic method to estimate insulin sensitivity; the frequently sampled intravenous glucose tolerance test (IVGTT) with minimal model analysis. This mathematical model couples two equations to assess insulin-mediated glucose disposal (87, 168, 169). When modified to include insulin, this method closely correlates to the glucose clamp. Without the insulin modification, the model is unable to produce meaningful results for insulin sensitivity in cases where insulin secretion is low, such as during T2D (87, 170, 171).

1.6 Measuring insulin sensitivity

Whilst the hyperinsulinaemic-euglycaemic clamp has been termed the "gold standard" procedure to assess insulin sensitivity (157), it is not without its practical limitations (87, 172). Its labour-intensive, time-consuming and expensive nature and the expertise required to conduct a glucose clamp make it challenging to apply in large cohort studies (87). In addition, the duration and intensity of the hyperinsulinaemia creates a non-physiological environment implying that using the clamp to measure insulin sensitivity may not reflect the human physiological response that would occur following a meal. Several simple surrogate estimates of insulin sensitivity have been derived from fasting (static state) or the oral glucose tolerance test (dynamic state), which include the homeostatic model assessment (HOMA) (173), quantitative insulin sensitivity check index (QUICKI) (171), oral glucose insulin sensitivity index (OGIS) (174) and the Matsuda index (175). All these models make assumptions about glucose homeostasis which may differ between different subjects. The models have been validated by comparing their results against clamp-derived measures.

Table 1: *A summary of the different experimental methods used to measure insulin sensitivity*

Method	Description	Strengths	Limitations
HEC	A direct measure of glucose disposal for a given insulin concentration using exogenous insulin and glucose intravenous infusions. Assessments are made during the basal and insulin stimulated steady state whereby the variation in blood glucose, insulin and the glucose infusion are minimal.	-When combined with isotopic tracers (which allow for a multi-compartment model), it can be used to assess endogenous glucose production, peripheral glucose disposal and whole-body lipolysis. -It creates a controlled hyperinsulinaemic plateau and is highly reproducible.	-Turns off physiological feedback loops and creates a supraphysiological environment. -Risk of hypokalaemia. -Time consuming due to the steady state requirement. -Labour intensive. -Invasive / high participant burden.
Frequently Sampled IVGTT with	The minimal model (MINMOD) is applied to glucose and insulin data from an IVGTT. There are two	-Can also be used to assess the acute insulin response and glucose effectiveness.	-An indirect assessment based on dynamic changes in glucose and insulin.

MINMOD	main equations that define the MINMOD; one describes glucose dynamics in a single compartment and the other describes insulin dynamics in another remote compartment. A modified frequently Sampled IVGTT, include an insulin or tolbutamide dose, is applied in participants known to be insulin resistant.	-Does not require a steady state and is less labour intensive than the HEC.	-Assumes glucose and insulin dynamics function in separate single compartments which is an oversimplification of glucose homeostasis. -Requires frequent sampling -The test is known to be less reliable in individuals with impaired glucose tolerance and T2D.
OGIS	It is an equation which has been developed based on mathematical modelling which was optimised to match HEC data. The equation uses glucose and insulin data derived from a 75g OGTT.	-Based on physiological response to oral glucose. -Can be applied to a large group of participants. -Requires only 3 sampling time points. -Can distinguish between glucose tolerance groups. -Can be used for 3 or 2 hour OGTTs.	-Assumes the relationship between glucose clearance and insulin is linear. -Describes glucose and insulin dynamics based on a single compartment. -Assumes a fixed value for endogenous glucose production.
Matsuda	An equation which has been developed as a composite measure of peripheral and hepatic insulin sensitivity based on glucose and insulin data derived from 75g OGTT. This is based on the understanding that endogenous glucose production is not suppressed by 100%.	- Based on physiological response to oral glucose. -Can be applied to a large group of participants. -Requires only 5 sampling time points. -Incorporates an assessment of hepatic insulin sensitivity.	-Endogenous glucose production is assumed to reflect product of fasting glucose and insulin -Assumes that whole-body insulin sensitivity during the OGTT is inversely proportional to the product of the mean plasma insulin and mean plasma glucose concentrations.
HOMA	A surrogate index for insulin sensitivity based a computer-solved model of insulin and glucose interactions. It requires postabsorptive conditions.	-Particularly sensitive in T2D where insulin levels are low and insufficient to maintain euglycemia. -Easily applied to large studies -Correlates well with hepatic insulin sensitivity.	-Does not reflect post-prandial insulin sensitivity
QUICKI	An index to assess insulin sensitivity based on postabsorptive glucose and insulin.	-Easily applied to large studies	-Does not reflect post-prandial insulin sensitivity

1.6 Measuring insulin sensitivity

1.7 The potential influencers of insulin resistance

There is a long-recognised link between adiposity and insulin resistance. Studies which suggest a causal relationship between excess adiposity and insulin resistance have been conducted in humans whereby overfeeding lean individuals with no prior connection to obesity or T2D reduces their insulin sensitivity. Assessing the effect of overfeeding on insulin sensitivity in controlled research settings has generally focused on acute short-term effects, as opposed to chronic overfeeding, due to ethical and resource limitations. An example of one of the longer term overfeeding studies measuring insulin sensitivity and body composition was authored by Johannsen *et al* (176). Johannsen and colleagues assessed 29 healthy men at an average age of 26; free from chronic metabolic disease, free from a history of eating disorders and reported to have a stable weight for at least 6 months prior. The participants were overfed by 40% of their energy requirement for 8 weeks and re-assessed. The participants exhibited an average weight gain of 7.6kg as well as a statistically significant increase in fat mass, percentage body fat, abdominal subcutaneous fat, visceral adipose tissue and intrahepatic fat. Insulin sensitivity was also assessed using a two-step hyperinsulinaemic-euglycemic clamp and showed an increase in hepatic and peripheral insulin resistance (176). The finding of increased fat and decreased insulin resistance has been echoed in other overfeeding studies in healthy participants (177, 178). Interestingly, other overfeeding studies using a very high dose of dietary fructose (179), and high-carbohydrate (HC) or high-fat (HF) overfeeding for 5 days (180, 181) showed no effect on insulin sensitivity. In these studies, there was no weight gain which may imply that it is the weight gain associated with overfeeding that is linked to the fall in insulin sensitivity.

Notably, not all individuals who are obese develop insulin resistance, they are often described to be metabolically healthy and at an energy balance (26, 182). A considerable amount of research has been conducted to identify and explain what may be influencing insulin resistance associated with obesity and T2D.

1.7.1 Visceral adipose tissue

Visceral adipose tissue (VAT) is defined as the adipose tissue which surrounds the intra-abdominal organs (183). Studies have shown that it is the rise in VAT independently, as opposed to general obesity, which predicts an increased risk of T2D and prediabetes (25, 26). Neeland and colleagues assessed 732 obese participants without T2D who were enrolled in the Dallas Heart Study; a multi-ethnic population based adult cohort, and followed up for a median period of 7 years (184). The participants underwent magnetic resonance imaging (MRI), dual-energy x-ray absorptiometry (DEXA) and other biochemical measures. 11.5% of participants developed T2D and whilst there was no difference in BMI, body fat % or abdominal subcutaneous adipose tissue (SAT) between those who did and did not develop T2D; VAT and liver fat % were significantly higher in those who progressed to T2D. Dividing the data into tertials also showed a significant trend for increasing T2D incidence with higher VAT, this trend was not found for SAT or total body fat. Finally, multivariable logistic regression modelling revealed VAT as one of the independent factors associated with the incidence of T2D and pre-diabetes (184). Studies assessing the pathophysiology of T2D have found a significant association between VAT and insulin resistance (182, 185, 186) which may explain the increased risk with increasing VAT. The accumulation of VAT is thought to be a marker of SAT dysfunction (185) as VAT is thought to form in response to

dysfunctional lipid handling in SAT. SAT has the unique ability to safely store lipids as triglycerides by increasing adipocyte number (adipogenesis/ hyperplasia) (187). However, during excess caloric intake and reduced energy expenditure, SAT accumulation is thought to reach its capacity and is unable to continue this function (188). Therefore, adipocyte hypertrophy occurs, and the adipocytes become pro-inflammatory, insulin resistant and unable to store fatty acids as depicted in figure 5. This processes is often described as unhealthy adipose tissue expansion. The large dysfunctional adipocytes become resistant to insulin and allow fatty acids to spill over into other depots which include the visceral cavity (VAT) (137, 189) as shown in figure 5. Overall, the inability of SAT to store fatty acids may increase fatty acids release which accumulates as VAT (141, 188, 190-193). The dysfunctional fat metabolism and change in fatty acid trafficking have been described in multiple distinct theories including the “spillover”, “adipose tissue expandability”, “lipid overflow” and “sick adipose tissue”, each describes processes which are likely to occur simultaneously and may foster VAT accumulation (194). Lipodystrophy is a human model which supports the potential for dysfunctional lipid handling in SAT, leading to VAT accumulation and metabolic dysfunction. Patients with lipodystrophy show low SAT, but high VAT and typically display high insulin resistance. In comparison, studies in humans with low VAT and high SAT show participants to be relatively insulin sensitive. Other experimental studies in animal models support the theory that VAT is more detrimental to insulin sensitivity than SAT (195).

Whilst individuals with high VAT have been described to be insulin resistant and present with dysfunctional glucose metabolism, the role of SAT and insulin resistance is less clear with the evidence from the overfeeding studies and the Dallas Heart study leading

1.7 The potential influencers of insulin resistance

towards VAT as the culprit and not SAT. In addition, Klein and colleagues have studied women with normal glucose tolerance and abdominal obesity; they assessed the effect of reducing abdominal subcutaneous adipose tissue (through liposuction) on insulin sensitivity assessed by a clamp and found no improvement in insulin sensitivity suggesting the lack of a relationship (196). However, there is some evidence to suggest that an increase in SAT is associated with a decreased risk of insulin resistance (186, 197). McLaughlin *et al*. assessed overweight and obese adults free from T2D who were stable in weight 3 months prior to their study visits. Participants underwent an insulin suppression test to assess insulin sensitivity and computerized tomography scans to assess VAT and SAT volume. They categorised participants as inulin sensitive or resistant based on previously published data. Using logistic regressions, it was found that each increment increase in VAT was associated with an 80% increased risk of being insulin resistant whereas each incremental increase in SAT was associated with a 42% decreased risk of insulin resistance. Therefore, the insulin sensitive phenotype may be associated with having less VAT and more SAT (198). This data implies that even though VAT only contributes towards 5-10% of total fat (199), an increase in VAT is negatively associated with insulin sensitivity. This link is further supported by a lack of insulin resistance in individuals with lower body obesity and the removal of abdominal subcutaneous adipose tissue having no effect on insulin resistance (200). Finally, the removal of VAT from obese rodent models has shown significant improvements in peripheral insulin sensitivity, using the hyperinsulinaemic-euglycemic clamp, which provides evidence for a causal association to insulin resistance (201, 202). Studies specifically assessing peripheral and hepatic insulin sensitivity have found a significant association with VAT in T2D (203-205).

1.7 The potential influencers of insulin resistance

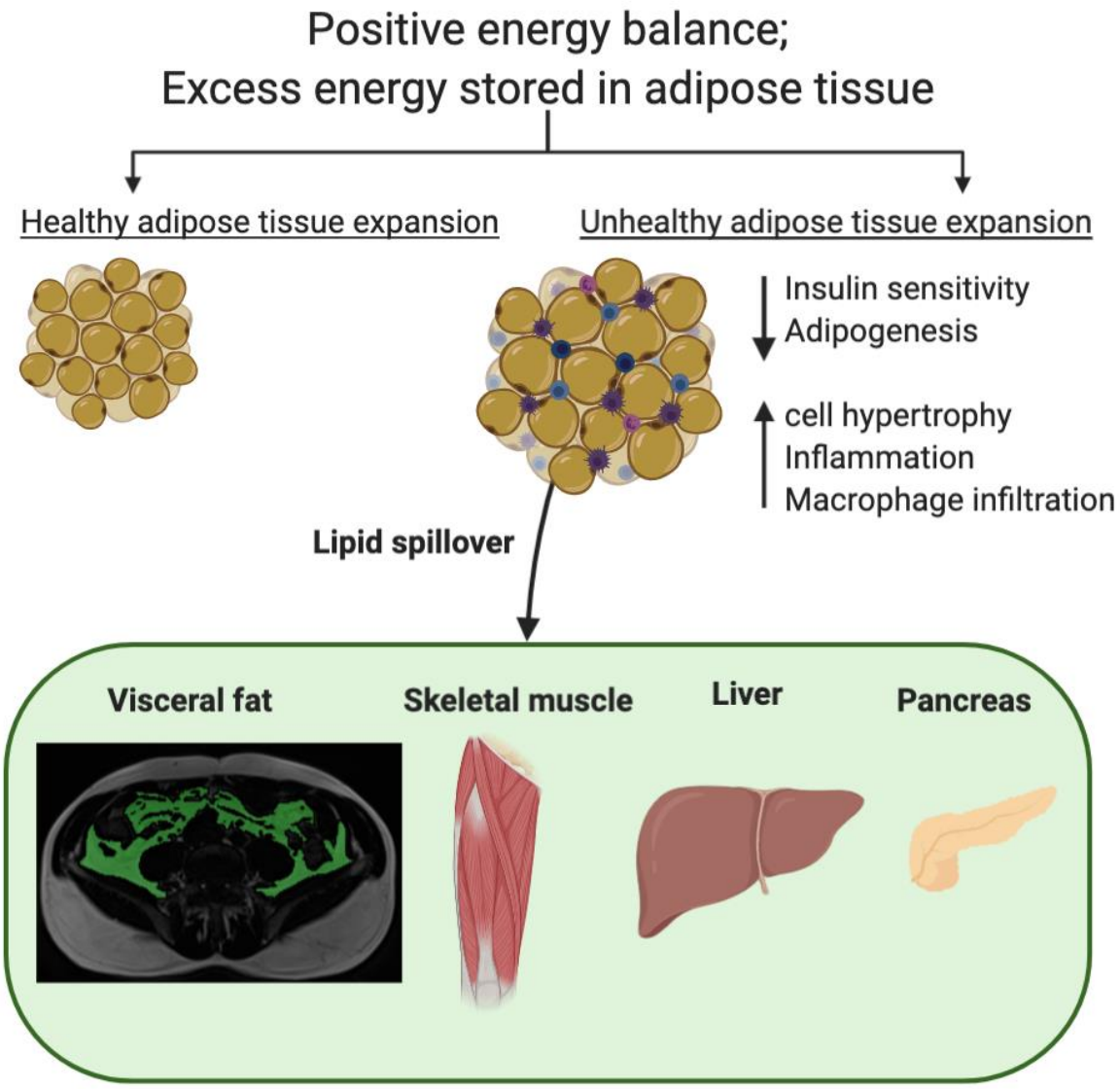


Figure 5: *Details for the changes which occur during unhealthy adipose tissue expansion in response to excess caloric intake and reduced physical activity. Dysfunctional lipid storing and adipose tissue remodelling may contribute to the accumulation of visceral and ectopic fat. Created with BioRender.com.*

1.7.1.1 Visceral adipose tissue and insulin resistance

Investigations into the potential mechanisms which may explain the link between VAT and insulin resistance implicate the secretion of adipose-specific cytokines (adipokines) and inflammatory cytokines from visceral adipocytes. The inflammatory cytokines include

interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α), which may potentially drain into the portal vein and then into the systemic circulation where they contribute to hepatic and peripheral insulin resistance; thus VAT can be described as a diabetogenic fat depot (182). These cytokines, particularly TNF-α, have been shown to decrease insulin signalling in the liver and muscle, which therefore increases hepatic glucose production and reduce peripheral glucose disposal, respectively (206-208). Leptin, an adipokine which impairs insulin sensitivity, has been shown to fall in studies where VAT is reduced and improvements in peripheral insulin sensitivity are shown (209, 210). This provides evidence to suggest that adipokines may mediate the association between VAT and insulin sensitivity. However, *in vivo* human studies to support the role of adipokines and inflammatory markers in insulin resistance are scarce. In addition to its inflammatory properties, VAT has also been described to be highly lipolytic, hence releases fatty acids which also end up in the peripheral circulation and may cause insulin resistance (194, 211). This process has been summarised in the "portal theory" which proposes that the highly lipolytic VAT drains free fatty acids and inflammatory cytokines into the portal vein, where they are delivered to the liver and systemic circulation which contributes to hepatic and peripheral insulin resistance, respectively (199, 212-214). The portal theory has been supported by research from multiple groups using molecular data (213) animal models (212) and human clinical studies (215). Another potential mechanism linking VAT with insulin resistance, which may work with or independently from the "portal theory", suggests that the accumulation of VAT is a signal for the accumulation of ectopic lipids which contribute to insulin resistance through lipotoxicity (182).

1.7 The potential influencers of insulin resistance

1.7.2 Ectopic fat

Ectopic fat is defined as the storage of triglycerides in non-adipose tissue (137). The tissue and organ-specific nature of this storage have been shown to associate with tissue and organ-specific dysfunctions. Concerning the development of T2D, longitudinal data support the notion that increased visceral and ectopic fat increases the risk of T2D (216). The accumulation of ectopic fat increases organ-specific insulin resistance through lipotoxicity (195). The organs of interest which have been found to accumulate ectopic fat and insulin resistance in T2D include the liver and muscle where it is termed intrahepatic lipid (IHL) and intramyocellular lipid (IMCL), respectively (134, 195, 217-219).

Similar to VAT, the mechanisms which are thought to lead to the development of ectopic fat centre around dysfunctional SAT lipid handling in response to a positive energy balance (excess caloric intake, reduced energy expenditure) (134, 137, 188, 220). As discussed in section 1.7.1, during the healthy state, subcutaneous adipocytes undergo hyperplasia to store triglycerides. During the positive energy balance, unhealthy SAT expansion is indicated by enlarged and overwhelmed adipocytes. They become pro-inflammatory, insulin resistant and unable to store fatty acids as triglycerides safely. This leads to a spillover of fatty acids into the visceral cavity as well as non-adipose organs such as the liver and muscle where they are stored as ectopic fat (figure 5). This deposition may occur simultaneously or at different points in the progression to T2D (134, 137, 188, 220). Overall, dysfunctional SAT leads to a redirection of triglycerides into VAT and non-adipose organs. Within the non-adipose organs during health, fatty acids are metabolised via beta-oxidation in the mitochondria for fuel. The inability of the mitochondrial function to increase and match the increased supply or impairments in the mitochondrial beta-oxidation, leads to

the formation of lipids intermediates (e.g. diacylglycerol). In these organs, the lipids promote organ-specific insulin resistance by impaired insulin action, thus T2D (137, 195, 221).

Liver fat is of particular interest in the T2D development because it is described to accumulate early in the pathogenesis of T2D; a high proportion of individuals with T2D have fatty liver (222). Increased IHL has also been associated with peripheral and adipose tissue insulin resistance in a large European cohort which provides further evidence for the role of IHL in the development of T2D (137, 223). Interestingly, IHL has been suggested to be equally or more detrimental to insulin resistance than VAT (224, 225). Other mechanisms outside of the "spillover", "adipose tissue expandability", "lipid overflow" and "sick adipose tissue" hypotheses have been explored to explain the formation of IHL. This includes the "portal" theory which is discussed in section 1.7.1 to explain the potential causal association between VAT and insulin resistance. The "portal" theory is also described to increase IHL deposition as fatty acids which drain from the lipolytic VAT through the portal vein contribute to the accumulation of IHL. Finally, the increased delivery of dietary fatty acids and glucose to the liver and fatty acids from adipose tissue lipolysis increases hepatic *de novo* lipogenesis, thus forming fatty acids which are esterified to triglycerides in the liver which may also be a mechanism for hepatic insulin resistance (220).

1.7.2.1 Hepatic fat and hepatic insulin resistance

Hepatic insulin resistance, defined as impaired insulin-mediated suppression of hepatic glucose production, increases with increasing IHL content (226, 227). A causal link for IHL and hepatic insulin resistance is implied from human interventions which reduce IHL and also show a decrease in hepatic insulin resistance (1, 228-230). The causal link between

IHL and insulin resistance is further supported by the ‘twin cycle hypothesis’ which has been developed to describe the development of T2D. It postulates, using longitudinal study data, that excess caloric intake with the backdrop of peripheral insulin resistance and hyperinsulinaemia increases ectopic deposition fat in the liver, which causes hepatic resistance (222).

Molecular studies which have assessed the effect of IHL on hepatic insulin sensitivity reveal that the accumulation of IHL increases levels of cytosolic hepatic diacylglycerol and other fatty acid metabolites which cause cellular dysfunction. Hepatic diacylglycerol is the final substrate in the synthesis of IHL. The hepatic diacylglycerol activates a protein kinase enzyme which ultimately causes hepatic insulin resistance. It does this by inhibiting the phosphorylation of the insulin receptor, thus preventing the downstream signalling cascade which leads to insulin-mediated suppression of hepatic glucose production. Therefore, hepatic diacylglycerols decrease glycogen synthesis and increase hepatic gluconeogenesis (1, 128, 134, 226, 231).

Whilst there is some evidence for a causal relationship between IHL and insulin resistance, it has been suggested that IHL is more of a marker rather than the cause of hepatic insulin resistance (232). Amaro and colleagues assessed overweight and obese participants with normal intrahepatic triglyceride, non-alcoholic fatty liver disease (NAFLD) and individuals with familial hypobetalipoproteinemia (FHBL); a genetic condition which leads to the accumulation of 3 times the intrahepatic triglyceride content found in healthy age and BMI matched individuals. The participants underwent a hyperinsulinaemic-euglycemic clamp to assess peripheral and hepatic insulin sensitivity, magnetic resonance imaging and magnetic resonance spectroscopy to assess VAT and intrahepatic triglyceride content (232).

1.7 The potential influencers of insulin resistance

There was no difference between the hepatic fat content in participants with NAFLD compared to those with FHBL however hepatic and peripheral insulin sensitivity was significantly greater in participants with FHBL. The participants with FHBL presented with similar peripheral and hepatic insulin sensitivity to healthy participants with normal hepatic fat content (232). These data suggest there is a disconnect between hepatic steatosis and insulin sensitivity and intrahepatic triglycerides may not cause resistance. It could also be interpreted that hepatic insulin resistance causes IHL deposition. Hepatic insulin resistance may increase hyperinsulinaemia which increases fatty acid uptake and triglyceride synthesis in SAT or ectopic depots (154, 233). In the liver, hyperinsulinaemia increases *de novo* lipogenesis, thus hepatic insulin resistance may increase IHL (134, 219) rather than IHL causing hepatic insulin resistance. Finally, studies have shown hepatic insulin sensitivity can improve independently of reducing IHL (234) and reducing IHL does not always increase hepatic insulin sensitivity (235, 236), suggesting that IHL is not the only contributor to hepatic insulin resistance or that in cases of sever steatosis, IHL content needs to reduce to normal levels in order to see an effect on hepatic insulin sensitivity.

1.7.3 Adipose tissue insulin resistance

In addition to visceral and ectopic fat, a resistance to the antilipolytic action of insulin in the adipose tissue has also been linked to peripheral and hepatic insulin resistance. As discussed in section 1.5.3, insulin has a potent action on adipocytes whereby it suppresses lipolysis. Resistance to this action results in inappropriately high rates of adipose tissue lipolysis and has been consistently been implicated with peripheral and hepatic insulin resistance, and therefore the progression to T2D (107, 141, 142, 153, 154, 233).

It is thought that one of the mechanisms linking excess lipolysis to insulin-resistant glucose metabolism is by increasing the availability of fatty acids (220, 237). This mechanism is supported by a large number of human studies which have either applied or removed fatty acids *in vivo* and shown reduced and increased insulin sensitivity to glucose metabolism, respectively (72, 135, 152, 197, 238). Whilst this mechanism appears promising, it has been suggested that the link between adipose tissue insulin resistance to lipolysis and peripheral insulin sensitivity is not a response of increased fatty acid availability, but rather the result of all tissues becoming resistant at the same time (239). In addition, increased availability of fatty acids can be derived from dietary intake or increased hepatic *de novo* lipogenesis (219), thus the mechanism which specifically links lipolysis to insulin resistance of glucose metabolism has yet to be concluded. Another proposed mechanism which links excess lipolysis with insulin resistance of glucose metabolism (peripheral and hepatic) is that increased lipolysis contributes to the formation of ectopic fat and VAT (135, 137, 240), which is associated with tissue-specific insulin resistance as discussed in section 1.7.1 and 1.7.2. The accumulation of ectopic fat and VAT have been described as part of the "spillover", "lipid overflow", "adipose expandability" and "portal" theories which identifies dysfunctional lipid handling in SAT as the central pathology. Overall the inability of SAT to store fatty acids combined with the resistance to insulin's suppression of lipolysis increases the release of fatty acids from the SAT which promote ectopic fat and VAT accumulation which may, in part explain the association between excess lipolysis with peripheral and hepatic insulin resistance to glucose metabolism (141, 188, 190-193).

1.7.3.1 Impact of fatty acid availability on hepatic insulin resistance

Hepatic insulin resistance, in particular, has been shown to be highly regulated by circulating fatty acids. A positive energy balance and an increase in lipolysis in response to adipose tissue insulin resistance increase circulating glycerol and fatty acids. This impairs insulin-mediated suppression of hepatic glucose production and therefore, increases hepatic glucose production. As discussed in section 1.5.2.1, fatty acids and glycerol can be taken up by hepatocytes as precursor/substrate molecules for gluconeogenesis. Fatty acids and glycerol therefore act as an extrahepatic signal which increases hepatic glucose production, and this is supported by *in vivo* evidence (107, 128, 135, 136, 241). Some investigators have suggested that this indirect mechanism of insulin resistance is more dominant than the direct action of insulin on the hepatocyte, and further research is required. However, it is accepted that FFA driven hepatic glucose production works independently to the direct effects of insulin on the hepatocyte (72, 128, 218, 220, 242).

1.7.4 Other influencers

Other factors which have been shown to influence insulin sensitivity include inflammation as described in the "spillover theory" (132, 243) and mitochondrial stress (1) /inflexibility during increased fatty acid and glucose availability (134). These influencers of insulin sensitivity are beyond the scope for this thesis but are to be acknowledged.

1.8 Indicators for an ethnic-specific pathophysiology of type 2 diabetes

As discussed in section 1.3.2, ethnicity is a risk factor for the development of T2D, particularly in individuals who identify as being of black ethnicity. Although there is a large

degree of heterogeneity within the black community, the majority of the literature in this section is based on African-Americans, sub-Saharan Africans and first generation black African-Caribbean's residing within the European diaspora. There are several distinctive characteristics shown in these black communities which indicate that an ethnic-specific process may occur in the development of T2D.

Prevalence and epidemiology data show from black communities in the UK (who identify as black African or Caribbean) and in the USA (who identify as either African-Americans, non-Hispanic black) are diagnosed with T2D at a lower age, have a lower BMI and lower central adiposity (waist circumference) at diagnosis (63-65, 67). Studies which have undertaken a more detailed assessment of body composition, show greater muscle mass in these black communities (244), lower visceral (245-255) and hepatic fat (246, 247, 252, 256-258) all of which are anti-diabetogenic characteristics. The progression of T2D increases with age, which is likely to reflect increases in adiposity (259, 260) which increases insulin resistance and T2D risk. The lower age, BMI and anti-diabetogenic body composition in black individuals diagnosed with T2D may suggest that the adiposity related risk occurs to a lesser extent in black communities.

In addition to the prevalence and body composition data, there is also evidence for ethnic differences in physiology which is associated with T2D. Hyperinsulinaemia is characteristic in the development of T2D; it is often described to result from the compensation mechanism for insulin resistance during the early stages of glucose intolerance (75). Populations of black ethnicity, who identify as black African, Caribbean, African-American and non-Hispanic black, are reported to present with exaggerated hyperinsulinaemia during the postabsorptive and insulin-stimulated state (251, 261-264). The

hyperinsulinaemia was initially thought to result from exaggerated beta-cell function and insulin secretion as a response to insulin resistance. This interpretation was based on a systematic review of studies assessing insulin sensitivity and the acute insulin response with a frequently sampled intravenous glucose tolerance tests in Africans, Caucasians, or East Asians participants across a range of glucose tolerances and included over 800 participants described as Africans (265). A more recent interpretation has identified reduced insulin clearance in black communities to potentially drive the hyperinsulinaemia and therefore, reduced clearance may be the initial defect in the black community (266). The reduced insulin clearance may also be a protective mechanism employed to preserve the pancreas from the beta-cell exhaustion which follows from over secretion of insulin. These observations also indicate that black populations may present with distinctive physiology which leads to T2D.

Having an unfavourable lipid profile is characteristic of metabolic syndrome and often associated with T2D (267). An unfavourable lipid profile is characterised by high levels of plasma triglycerides (within VLDLs and low-density lipoproteins (LDL)) which have been associated with a higher risk of atherosclerosis and cardiovascular disease (268) and less high-density lipoproteins (HDL) which have been associated with a lower risk for cardiovascular disease (269, 270). A favourable lipid profile is characterised as lower circulating plasma triglycerides (contained in VLDL and LDL) but higher HDL. The cardioprotection from HDL may result from the HDL cholesterol-lowering capacity via the reverse cholesterol transport hypothesis, although these data on this are not conclusive (270, 271). Whilst the pathophysiology to explain the dyslipidaemia observed in T2D has not been fully described, insulin has been shown to inhibit VLDL production by reducing circulating fatty acids which are required as a substrate for VLDL production (241). Insulin also

promotes the clearance of circulating VLDLs by increasing the activity of lipoprotein lipase in adipose tissue, as discussed in section 1.5.2 and 1.5.3 (272, 273). Therefore, a resistance to insulin, which occurs during the progression to T2D, is associated with increasing VLDL and an unfavourable lipid profile and this has been shown precede the development of overt T2D (273). In terms of ethnicity, black communities are consistently reported to display a cardioprotective lipid profile compared to their white counterparts in all glucose tolerance groups and when matched for BMI (274-277). They repeatedly present with lower triglycerides and some USA based studies show that the African-Americans and non-Hispanic black participants present with higher HDL compared to European-Americans, although this is not found in black south Africans suggesting the HDL characteristics may be geographically-specific (278). Explanations for the potential causes of the cardio protective lipid profile in black communities have implicated ethnic differences in lipoprotein lipase activity, which clears triglycerides, and hepatic lipase activity, which clears HDL. Data from black participants shows higher lipoprotein lipase activity and lower hepatic lipase activity and gene expression which may explain the ethnic differences in lipid profile (276, 279-281). What is particularly interesting with respect to black communities is that the cardioprotective profile occurs in the presence of insulin resistance (279). Given that insulin resistance is associated with dyslipidaemia, this creates a paradox and implies the pathophysiology of insulin resistance and therefore T2D, may be ethnically distinct.

As discussed in section 1.6, there are a number of methods to assess insulin sensitivity, including various surrogate indices which estimate insulin sensitivity during the postabsorptive state or an oral glucose tolerance test. Population based studies comparing insulin sensitivity using the homeostasis model assessment (HOMA) in black and white

populations ,covering a range of BMIs, glucose tolerances and health statuses (including those with hepatic steatosis), consistently show black communities to have greater insulin resistance, particularly in women (256, 282-284). The IVGTT has also been utilised extensively in black and white participants and pronounced insulin resistance in black participants have predominantly been reported. These studies have assessed participants with a range of glucose tolerances, although far more studies have been conducted in participants with normal glucose tolerance. In addition, the pronounced insulin resistance in black communities has also been reported and adolescents and the ethnic difference in insulin sensitivity persist when matching for body composition measures (265, 285, 286). The first study to assess insulin sensitivity in black and white participants using the IVGTT with minimal model analysis was published in 1993. 22 black and 20 white normal glucose tolerant obese women were matched for age, BMI and menopausal status. The insulin sensitivity index results suggested that there was no ethnic difference in insulin sensitivity (287). Shortly after, Osei and colleagues also assessed normal glucose tolerant black and white Americans across a range of BMIs however, the sample size was larger (black =32, white = 30) and included men. Their published findings showed between 35 to 52% lower insulin sensitivity in black compared to white participants following an IVGTT (261, 288). Some of the most compelling data have been derived from 'The Insulin Resistance Atherosclerosis Study'(IRAS) which used the IVGTT with minimal modelling to assess insulin sensitivity in over 400 African-Americans and over 600 non-Hispanic white participants. The authors report 29 to 41% lower insulin sensitivity in African-Americans compared to non-Hispanic white participants without T2D (289) and no ethnic difference in those with T2D (290). When participants within the IRAS were pooled, there was no ethnic

difference in age or glucose tolerance status however BMI was significantly higher in Africans-Americans compared to the non-Hispanic white participants. Insulin sensitivity remained lower in Africans-Americans compared to non-Hispanic white participants (291). Since then, there has been an abundance of reports for higher insulin resistance in black participants without T2D compared to their white counterparts (248, 255, 292-298). However, those with T2D appear to have less pronounced differences in insulin sensitivity (252, 265) suggesting that impairments occur early on in the progression to T2D. The suggestion of an early defect of insulin sensitivity in black compared to white participants is further supported by reports of increased insulin resistance in healthy black compared to white adolescents (297, 299-302). Whilst the narrative is driven towards black communities being more insulin resistant from multiple research studies, the literature is not conclusive, with some studies finding no difference from the IVGTT (252, 287, 303, 304). Studies which have utilised glucose clamps, termed the "gold standard" method for assessing insulin sensitivity, also produce inconsistent results which are discussed in more detail in section 1.9. It is currently thought that one of the potential influences of insulin resistance during the development of T2D is the accumulation of visceral fat, ectopic fat and excess lipolysis as discussed in section 1.7 (182, 185, 186, 195, 216). In black populations, the pronounced insulin resistance is observed in the presence of lower visceral and ectopic fat. This does not align with the views on T2D pathophysiology and further suggests that an ethnic distinction in T2D pathophysiology may be present.

Overall these distinctive characteristics suggest there may be an ethnic-specific pathophysiology of T2D, particularly relating to insulin sensitivity. Many research groups

1.8 Indicators for an ethnic-specific pathophysiology of type 2 diabetes

have conducted comparisons in black and white adolescents to understand the ethnic-specific pathophysiology; however, this thesis will focus on data from adults.

1.9 Insulin sensitivity and black ethnicity

Assessments of insulin sensitivity based on surrogate estimates have been shown to associate with clamp derived measures of insulin sensitivity in black adults (305, 306). However, Pisprasert and colleagues have more recently provided data which shows that in a group of African Americans and European Americans with the same insulin sensitivity (total glucose disposal) measured from the clamp, surrogate estimates show African Americans to be more insulin resistant (307). This finding is likely to be the result of pronounced hyperinsulinaemia, which is consistently reported in black communities, as discussed in section 1.8 (251, 261-264). Hyperinsulinaemia may lead to an overestimation of insulin resistance from methods based on mathematical modelling of glucose and insulin dynamics such as surrogate indices (307). Pisprasert and colleagues do show correlations between the clamp and surrogate indices in black participants. However, in black men, the relationship was not consistently significant. The latter finding agrees with a study in UK based afro-Caribbeans who also found no significant association between HOMA-IR (a measure of fasting insulin sensitivity based on fasting glucose and insulin concentrations) and insulin sensitivity measured from the clamp (308). Overall these studies suggest that using surrogate estimates of insulin sensitivity or resistance in black populations can lead to misleading conclusions and should be evaluated with caution (307, 309). Pisprasert and colleagues also suggest that the hyperinsulinaemia in black communities may affect insulin sensitivity measured from an IVGTT with minimal model analysis (307). The minimal model analysis

is also based on mathematical modelling of ambient glucose and insulin concentrations. No studies have compared the IVGTT with minimal modelling to the clamp in black participants, therefore it is not conclusive as to whether the hyperinsulinaemia overestimates insulin resistance in black participants. “Gold standard” highly sensitivity tools to assess insulin sensitivity in black and white adults are essential to understand ethnic differences without the potential confounding of the hyperinsulinaemia (310). This thesis explores the potential ethnic-specific pathophysiology and is focused on *in vivo* assessments of whole-body and tissue-specific insulin sensitivity in black compared to white adults.

1.9.1 Whole-body insulin sensitivity and black ethnicity

Whole-body insulin sensitivity is generally used to describe the combined effects of peripheral glucose uptake and suppression of hepatic glucose production in response to insulin and is often represented as the M value or total glucose disposal. Studies in black and white adults which have assessed whole-body insulin sensitivity during the hyperinsulinaemic-euglycaemic clamp have shown inconsistent results. This may be due to factors such as sex, glucose tolerance, BMI and family history which may influence ethnic specific findings.

The majority of studies assessing whole body insulin sensitivity using the hyperinsulinaemic-euglycaemic clamp in populations of black and white ethnicity have focused on women, all of which do not have T2D. The studies are based on obese and overweight South African and African-American women with an average BMI ranging from 32 - 26 kg/m^2. The sample sizes ranged from 9 to 10 black women and 10 to 26 white women. The black and white participants were matched for BMI and age however whole-body insulin

sensitivity was lower in black compared to white women. The mean difference in whole-body insulin sensitivity was between 40% and 60% lower in obese black women compared to obese white women, due specifically to lower non-oxidative glucose disposal (glycogen storage) (245, 251, 311). In comparison, studies focused solely on men are absent and have not been a focus for any research groups. That being said, studies which have recruited both men and women but analysed the data by sex provide some evidence for potential ethnic differences in whole body insulin sensitivity in men. A single study found 25% greater whole-body insulin sensitivity in non-diabetic black men compared to non-diabetic white men all of whom where similar in BMI with an average BMI of 29.0 kg/m^2 (312). All other studies which have recruited both sexes show no ethnic difference in whole-body insulin sensitivity (307, 312-318). These studies contain sample sizes of over 30 and include participants with a range of BMIs and glucose tolerances. However, the black and white participants were not consistently matched for BMI such that black participants were heavier which may confound results and make interpretations more complex considering the influence of adiposity, particularly central adiposity, on insulin sensitivity.

A more recent focus, driven by the Pathobiology of Prediabetes in A Biracial Cohort (POP-ABC) study, has been on the impact of family history and ethnicity. This was a mixed sex study recruiting participants across a range of BMIs. They have published data from clamps showing that in the absence of family history, male and female black participants have lower insulin sensitivity (the mean is 37% lower) compared to white participants (319). The obesity status of participants was not reported however the same group have published another report showing that when a positive family history was present, there was either a trend towards or significantly lower insulin sensitivity in black participants compared to their

white counterparts (319-321). In these reports, similar to some of the studies discussed previously, participants were not matched for BMI such that the black participants were more obese which may confound the insulin sensitivity findings.

Overall data from hyperinsulinaemic-euglycaemic clamp studies are inconsistent with most evidence pointing towards either lower insulin sensitivity in black compared to white participants or no ethnic difference in whole-body insulin sensitivity. Some of these interpretations are based on study populations which are not matched for BMI, which may confound results and the impact of this is not clear. In addition, most of the evidence points towards black women being more insulin resistant; however, studies in black and white men are scarce. Finally, an understanding of whole-body insulin sensitivity in each glucose tolerance group in isolation is limited- most studies assess NGT and IGT collectively or T2D alone.

1.9.2 Peripheral insulin sensitivity and black ethnicity

Peripheral insulin sensitivity is primarily an assessment of insulin-mediated skeletal muscle glucose disposal. The first study to use a glucose isotopic tracer to assess peripheral glucose disposal in lean black and white women showed 26% lower insulin sensitivity in black compared to white women. This difference was observed during the low and high dose insulin infusion in a population of participants well matched from BMI and age. In this study, participants were healthy, lean and young with an average BMI of 22.7 kg/m^2 and an average age of 24 years (322). By using indirect calorimetry, DeLany *et al.* were able to ascertain that the ethnic difference in peripheral insulin sensitivity was the result of lower non-oxidative glucose disposal as opposed to ethnic differences in glucose oxidation (322). This agrees

with *Ryan et al.,* who also found lower non-oxidative glucose disposal in black women but no difference in glucose oxidation (251). Goedecke and colleagues are the only other authors to assess peripheral insulin sensitivity in black and white women. They assessed peripheral insulin sensitivity in obese non-diabetic South-African and white participants matched for age and BMI, although fat percentage was higher in the black women. The study included participants with impaired glucose tolerance which was not equally distributed by ethnic group however, the authors completed a subgroup analysis to confirm that this did not alter their findings of no ethnic difference in peripheral insulin sensitivity (323). In addition to comparing peripheral insulin sensitivity in white and black women, Goedecke and colleagues also assessed the relationship with VAT. As discussed in section 1.7.1, VAT has consistently been associated with insulin sensitivity and increasing VAT accumulation may decrease insulin sensitivity. Goedecke and colleagues found no significant relationship between peripheral insulin sensitivity and VAT in black women, but a significant negative in white women. This suggests that having high VAT is not indicative of insulin resistance or having low VAT is not indicative of insulin sensitivity in black women. Whether these findings can be extrapolated to men of different glucose tolerances and BMI statuses is unknown.

1.9.3 Hepatic insulin sensitivity and black ethnicity

Hepatic insulin sensitivity is primarily used to describe insulin-mediated suppression of endogenous glucose production. The investigations into hepatic insulin sensitivity using glucose isotope tracers in black and white populations show inconsistent results which may be due participant characteristics such as sex and obesity, or due to the method used to assess hepatic insulin sensitivity, i.e. basal or insulin stimulated assessments.

Chung *et al.* studied 24 obese black and 22 obese white women with normal glucose tolerance and pre-diabetes who were matched for age and BMI which was an average of 37 years and 32.5 kg/m^2, respectively. They found no ethnic difference in basal hepatic insulin sensitivity between black or white individuals calculated based on the inverse of the product of basal endogenous glucose production and insulin (246). However, Goedecke *et al.* studied 15 obese black and 15 obese white south African women with normal glucose tolerance and pre-diabetes who were matched for age and BMI which was an average of 36 years and 36.6 kg/m^2, respectively. They found that insulin stimulated hepatic insulin sensitivity was over three times greater in black compared to white women (323). In opposition, Ellis and colleagues studied 23 African-American and 30 European American women across a range of BMIs (from 18.7 to 38.6 kg/m^2). The women were normal glucose tolerant with an average age of 25 years; they were recruited to be matched for age and BMI. Their data showed that mean basal hepatic insulin resistance was 18% higher in black compared to white women (296). In lean black and white women with normal glucose tolerance at an average age of 24 years, DeLany and colleagues present data showing no ethnic difference in basal or insulin stimulated hepatic insulin sensitivity (322). Finally, a single study authored by Stefan and colleagues recruited and pooled both sexes. The participants were normal glucose tolerant and there were no ethnic differences in age (an average of 31 years) or BMI (an average of 30 kg/m^2). In their analyses they showed no ethnic difference in insulin stimulated hepatic insulin sensitivity (315). Overall, the literature shows no consensus as to whether hepatic insulin sensitivity is similar, lower or higher in black compared to white populations.

As discussed in sections 1.7, VAT and IHL have been shown to associate with hepatic insulin sensitivity, with some evidence for a causal link. Studies in obese women have

assessed these relationships in black and white women. They show a significant association between basal hepatic insulin sensitivity and IHL in both ethnic groups (246). However, insulin stimulated hepatic insulin sensitivity only associated with IHL and VAT in black but not white women (323).

To summarise, studies in obese women provide evidence for a significant relationship between VAT and IHL in black women however, whether this can be extrapolated to men has yet to be determined.

1.9.4 Adipose tissue insulin sensitivity and black ethnicity

Adipose tissue insulin sensitivity to lipolysis can be assessed by quantifying the insulin-mediated suppression of lipolysis during an insulin clamp. A single study has used a glycerol isotopic tracer to assess adipose tissue insulin sensitivity in obese black and white women during insulin stimulation (304). *Albu et al.* studied 16 obese African-American and 13 obese non-Hispanic white participants free from T2D who were matched for age (35 years on average) and BMI (on average 35.9kg/m^2). They show significantly greater adipose tissue insulin sensitivity in obese black compared to white women. The insulin infusion provoked a 60% suppression of lipolysis in black women compared to a 40% suppression in white women (304). In comparison, a more recent study in obese women with an average BMI of 32.5 kg/m^2 authored by Chung *et al.* showed no ethnic difference in adipose tissue insulin sensitivity which was assessed during the basal state; participants were matched for BMI (246). The conflicting results may reflect differences in the method used to determine adipose tissue insulin sensitivity or VAT content which is of importance because VAT is highly lipolytic and an indicator of metabolic dysfunction (324).

No other studies have assessed adipose tissue insulin sensitivity in black and white populations. However, basal lipolysis in healthy women free from T2D matched for BMI and age has been shown to be similar in the presence of similar basal insulin levels which may imply that there are no ethnic differences in adipose tissue insulin sensitivity (303). In contrast, other studies in obese women who were also matched for BMI have shown lower basal lipolysis in African-American black compared to white women in the presence of similar insulin levels, which suggests higher adipose insulin sensitivity (252, 325). Overall, data are inconsistent, and there is scarcity of such studies in men.

Adipose tissue insulin sensitivity has been implicated in peripheral and hepatic insulin resistance as well as ectopic fat accumulation, as discussed in sections 1.7.3. No study has assessed this in black populations who unexpectedly, display insulin resistance in the presence of lower ectopic fat. A single study has shown a significant relationship between IHL and adipose tissue insulin resistance in black and white participants(326). This may suggest that adipose tissue insulin resistance is linked to hepatic insulin resistance through ectopic fat accumulation. However, the authors assessed adipose tissue insulin resistance using a surrogate index of adipose tissue insulin resistance and the participants included a mix of participants with and without T2D and non-alcoholic fatty liver disease thus, whether this finding can associated to T2D physiology cannot be concluded (326).

1.9.5 Insulin sensitivity and black ethnicity summary

To conclude, although the narratives in the literature have implicated black populations to have greater insulin resistance compared to white populations, the studies which assess and compare whole-body and tissue-specific insulin sensitivity from the clamp

and/or isotopic tracers produce conflicting findings and have primarily been conducted in women without T2D.

1.9 Insulin sensitivity and black ethnicity

1.10 Aims

This thesis aims to determine if there are ethnic differences in whole-body and tissue-specific insulin sensitivity between black west African (BAM) and white European men (WEM) at three stages of glucose tolerance: normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or early type 2 diabetes (T2D). It also aims to assess if there are ethnic differences in the relationship between insulin sensitivity and potential influencers of insulin sensitivity.

Black west African men have been chosen to reduce the heterogeneity within the black participants derived from factors such as genetics, behaviour and cultural factors, discussed in section 1.3.2.

1.10.1 Objectives

1. To quantify whole-body insulin sensitivity in BAM and WEM.
2. To quantify peripheral insulin sensitivity in BAM and WEM.
3. To quantify hepatic insulin sensitivity in BAM and WEM.
4. To quantify adipose tissue insulin sensitivity to lipolysis in BAM and WEM
5. To compare the associations between peripheral and hepatic insulin sensitivity with visceral fat, hepatic fat and adipose tissue lipolysis.

Each of the above will be performed in three glucose tolerance groups: NGT, IGT and early T2D, matched within glucose tolerance groups for BMI.

Chapter 2: Materials and Methods

2.1 Soul-DeEP study

The data analysed in this thesis have been collected as part of a cross-sectional observational study, the South London Diabetes and Ethnicity phenotyping (Soul-DeEP) study (327). This project was able to assess and compare metabolic functions and body composition parameters related to type 2 diabetes (T2D) in black west African (BAM) and white European men (WEM) with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and T2D. The primary aim of the Soul-DeEP study was to investigate the hypothesis that early pronounced peripheral insulin resistance in black men, leads to an early and exaggerated compensatory insulin secretion, that ultimately fails and drives the development of early-onset T2D in the BAM population. The study was powered to detect an ethnic difference in insulin secretory function; secondary outcomes focused on quantifying tissue-specific insulin sensitivity and fat depots to generate an all-encompassing view of potential ethnic differences in the pathophysiology of T2D between BAM and WEM. A schematic for the overall Soul-DeEP study design is shown in figure 6. The participants attended 4 separate visits following their screening appointment however, only data collected from visits C and D have been discussed as part of this thesis.

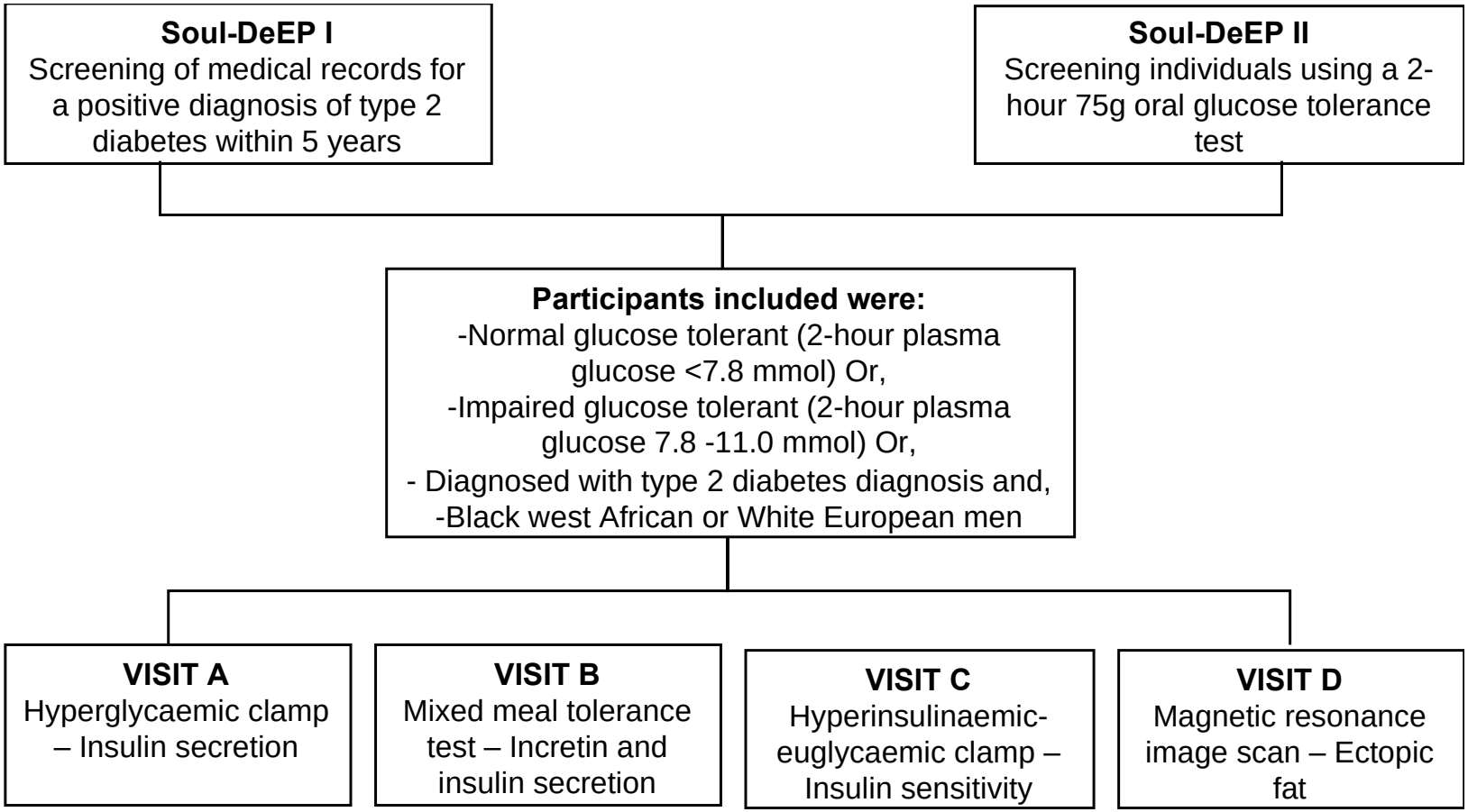


Figure 6: *Soul-DeEP study design schematic*

2.2 Participants

The Soul-DeEP study was divided into two phases (Soul-DeEP I & II) based on the recruitment of men with T2D in the first phase, then combining the recruitment of IGT and NGT in the second phase. Data collection for the former took place between April 2013 to January 2015 and data collection for the latter took place between April 2016 and May 2018. Participants were either self-referred from advertisements in newspapers, social media, the King's College London university staff and students community, research study websites, local colleges, religious groups, community groups, local business, word of mouth or were identified as high risk based on their glycated haemoglobin (HbA1c) or diagnosed with T2D for <5 years by their primary care practice in south London. All participants provided informed consent prior to any study procedures and the study was approved by the London Bridge National Research Ethics Committee (approval no. phase I; 12/LO/1859, phase II 15/LO/1121).

As discussed in chapter 1 (section 1.10), this study is focused on men. Men of black west African (BAM) and white European, (WEM) ethnicity between the ages 18 to 65 were invited to participate. Participant inclusion criteria included; a BMI range from 25 – 35 kg/m^2 for Soul-DeEP I and 25 – 40 kg/m^2 for Soul-DeEP II, NGT as defined by a 2-hour plasma glucose of <7.8 mmol/l during a 75g oral glucose tolerance test, or IGT as defined by a 2-hour plasma glucose of 7.8-11.0 mmol/l during a 75g oral glucose tolerance test, or a diagnosis of T2D <5 years and being treated with either metformin or lifestyle changes. Fasting plasma glucose was not used to define participant glucose tolerance status. Participants were excluded if they were being treated with thiazolidinedione, SGLT-2 inhibitors, insulin, chronic oral steroids, beta-blockers, any other medications known to affect

the study outcomes or if they had constraints to the magnetic resonance imaging protocol (MRI) such as a metal plate/implant/prosthesis or were unable to comply with the study protocol.

Eligibility was assessed during a comprehensive screening visit which took place at King's College Hospital. Ethnicity was self-declared by selecting either white British, white European, black west African or black other. The countries of origin used to characterise west Africa were based on the United Nations Statistics Division (UNSD) (328) and includes:

- Benin
- Burkina Faso
- Cabo Verde
- Côte d'Ivoire
- Gambia
- Ghana
- Guinea
- Guinea-Bissau
- Liberia
- Mali
- Mauritania
- Niger
- Nigeria
- Saint Helena
- Senegal
- Sierra Leone
- Togo

The countries of origin used to characterise European were also based on the UNSD however, the southern Europe geographical region was excluded:

- Belarus
- Bulgaria
- Czechia
- Hungary
- Poland
- Republic of Moldova
- Romania
- Russian Federation
- Slovakia
- Ukraine
- Åland Islands
- Channel Islands
- Denmark
- Estonia
- Faroe Islands
- Finland
- Iceland
- Ireland
- Isle of Man
- Latvia
- Lithuania
- Norway
- Svalbard and Jan Mayen Islands

2.2 Participants

- Sweden
- United Kingdom of Great Britain and Northern Ireland
- Austria
- Belgium
- France
- Germany
- Liechtenstein
- Luxembourg
- Monaco
- Netherlands
- Switzerland

Self-declared ethnicity was confirmed by parental and grandparental birthplace. Anthropometric measurements and fasting blood samples were also taken to assess eligibility. Weight and height were measured in light clothing and whilst wearing no shoes to determine BMI. Waist circumference was measured at the mid-point between the lower margin of the last palpable rib and the top of the iliac crest in line with the WHO recommendations (329). Screening blood samples were taken at fasting to assess HbA1c, confirm normal kidney and liver function, the absence of sickle cell (although the trait was permitted) and a normal full blood count. In addition to the screening safety blood tests, participants with T2D during Soul-DeEP I were screened for antibodies and were excluded if they tested positive for the auto-antibodies; anti-insulin, anti-glutamic acid decarboxylase or anti-A2. Family history of type 2 diabetes (within first degree relatives and extended family), past medical history (including known allergies), smoking and alcohol intake habits (including frequency and preferences) and triplicate seated blood pressure measurements (obtained from the non-dominant arm) were also documented at the screening visit.

2.3 Procedures

In preparation for the metabolic assessments, participants were instructed to fast from 10 pm the evening preceding the assessment (≥10 hours), refrain from alcohol for 24 hours

and strenuous physical activity for 48 hours, avoid smoking the morning of the visit and to ensure their evening meal contained a portion of carbohydrate. Patients with T2D were instructed to ensure approximately 50% of their energy intake came from carbohydrate spread evenly throughout the day and their evening meal contained no more than 30% of daily carbohydrate consumed in the evening meal. However, the research team did not scrutinise this and thus, it was not requested in the second phase of the study involving participants with NGT and IGT.

2.3.1 Screening

NGT and IGT status was confirmed by a 75g oral glucose tolerance test using the WHO cut-off points. The OGTT procedure began with the insertion of a cannula into the antecubital fossa vein in the arm for blood sampling at fasting (timepoints -10 and 0 minutes) then 10, 30, 60 and 120 minutes after a single 75g anhydrous oral glucose load. 2-hour plasma glucose <7.8mmol/l defined NGT, plasma glucose ≥ 7.8 and <11.1mmol/l defined IGT based on the WHO criteria (6). Participants were selected to be similar in age and BMI within each glucose tolerance group.

2.3.2 Hyperinsulinaemic-euglycaemic clamp

Whole-body and tissue-specific insulin sensitivity were assessed using a two-step hyperinsulinaemic-euglycaemic clamp with stable glucose and glycerol isotopic tracer infusions. As discussed in chapter 1 (section 1.6), it has been termed the optimal *in vivo* assessment of insulin sensitivity to glucose homeostasis and provides a quantitative assessment of total glucose disposal at a given insulin concentration. This *in vivo* assessment

of insulin sensitivity manipulates the glucose and insulin continuous feedback loops by creating a hyperinsulinaemic environment at a steady state. The glucose infusion required to maintain euglycaemia during hyperinsulinaemia is an assessment of total glucose disposal and therefore insulin sensitivity (89, 156). When combined with stable isotope tracers, which allow for direct measurements of substrate appearance and disappearance from the circulation, tissue-specific measurements of insulin sensitivity can be derived from the basal and insulin-stimulated state of the clamp (159). Stable isotopic tracers are chemically identical to their tracee but, differ in their molecular weight due to the number of neutrons they contain, making them distinct and quantifiable using mass spectrometry. In this study, deuterated glucose ([6,6-$^{2}H_{2}$]-glucose) and glycerol ([$^{2}H_{5}$]-glycerol) were used at a low concentration to prevent a significant imbalance in tracee kinetics which is discussed in more detail in section 2.6.2.

Upon arrival at the Clinical Research Facility of King's College Hospital, participants were weighed in light clothing with an empty bladder on digital scales or a body composition analyser (Tanita MC780MA) where available, to determine body weight, fat-free mass (FFM) and body surface area (BSA, using the Mosteller formula; square root of the height (cm) multiplied by the weight (kg) divided by 3600) for infusion calculations and insulin sensitivity indices. To begin the overall procedure, a cannula was placed into a vein on the dorsum of the hand in a retrograde fashion. The hand was kept in a 55° hand-warming unit to mimic arterialised sampling (330). Duplicate baseline samples were taken to determine naturally occurring background [6,6-$^{2}H_{2}$]-glucose and [$^{2}H_{5}$]-glycerol isotope enrichment before the basal phase commenced. An infusion cannula was inserted into the antecubital fossa vein of the contralateral arm for infusions of insulin (Actrapid, Novo Nordisk,

Bagsvaerd, Denmark) bound to albumin in 4% autologous blood/saline, 20% (wt/vol) dextrose, [6,6-$^{2}H_2$]-glucose and [$^{2}H_5$]-glycerol tracers (CK Gases, Cambridgeshire, UK). The basal phase of the hyperinsulinaemic-euglycaemic clamp was assessed from time -120 to 0 minutes and was initiated with a primed (2.0 mg/kg), continuous infusion (0.02 mg/kg^{-1} min^{-1}) of [6,6-$^{2}H_2$]-glucose and a primed (0.12 mg/kg), continuous infusion (0.0067 mg/kg^{-1} min^{-1}) of [$^{2}H_5$]-glycerol (234). A steady-state isotopic enrichment and saturation is achieved after 90 minutes, whereby the rate of tracer flux is in equilibrium with that of the tracee (160, 331). Blood samples were drawn at -30,-20,-10 and 0 minutes to determine basal assessments. The first step (low dose insulin) of the two-step hyperinsulinaemic-euglycaemic clamp began with a primed and continuous insulin infusion (10mU/m^{2}BSA/min) which was maintained for 120 minutes. The final 30 minutes of the low dose insulin infusion was defined as the steady-state period, whereby glucose appearance equals glucose disposal. Blood samples drawn during this time were used to assess hepatic and adipose tissue insulin sensitivity. For the second step (high dose insulin) of the two-step hyperinsulinaemic-euglycaemic clamp, the [$^{2}H_5$]-glycerol isotope infusion was terminated and the continuous insulin infusion was primed and raised to 40mU/m^{2}BSA/min for 120 minutes. The final 30 minutes of the high dose insulin infusion was defined as the steady-state period, whereby glucose appearance equals glucose disposal. Blood samples drawn during this time were used to assess whole-body and peripheral insulin sensitivity (156, 332). Euglycaemia was defined as 5 mmol/l and was maintained during both steps using a 20% dextrose infusion. The dextrose was enriched with [6,6-$^{2}H_2$]-glucose (8 mg/g glucose at low-dose insulin and 10 mg/g at high-dose insulin) to maintain a constant tracer-to-tracee ratio (333). The dextrose infusion was given at a variable rate, determined by the clinical research investigator, based

on plasma glucose readings taken every 5 minutes using an automated glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Yellow Springs, OH, USA). Samples were taken at 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230 and 240 minutes for assessment of plasma glucose, insulin and isotopic enrichment. The hyperinsulinaemic-euglycaemic clamp sampling and infusion protocol are detailed in figure 7, and an example of the graphs produced during the procedure to influence the dextrose infusion rate decision is shown in figure 8.

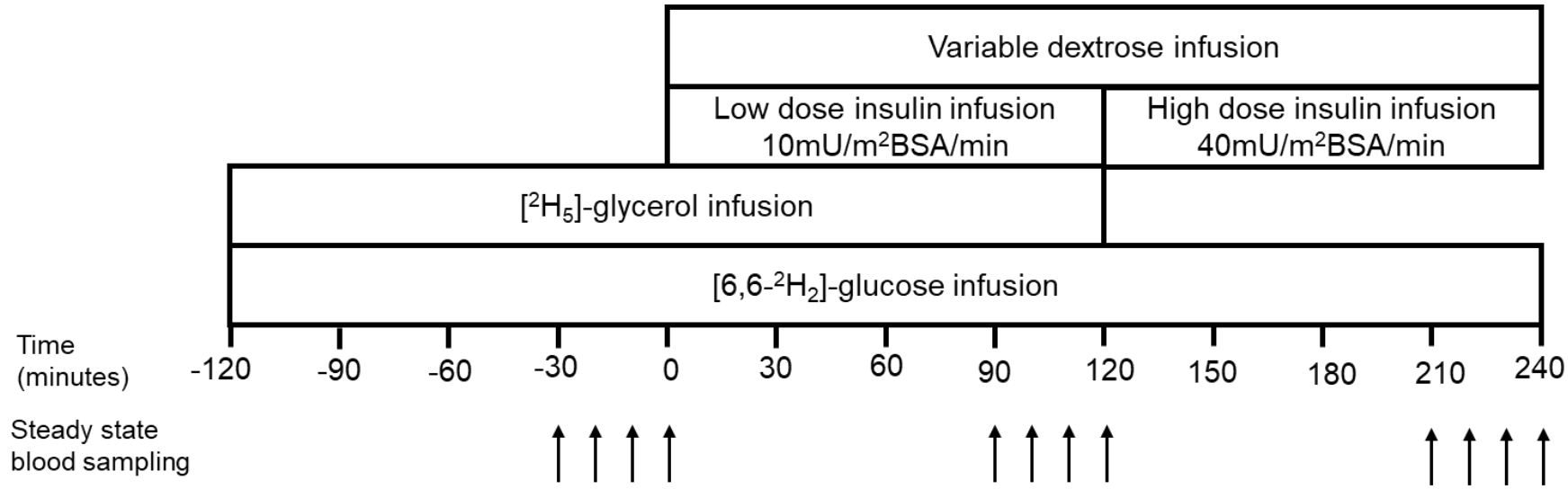


Figure 7: *A schematic of the hyperinsulinaemic-euglycaemic clamp protocol and sampling*

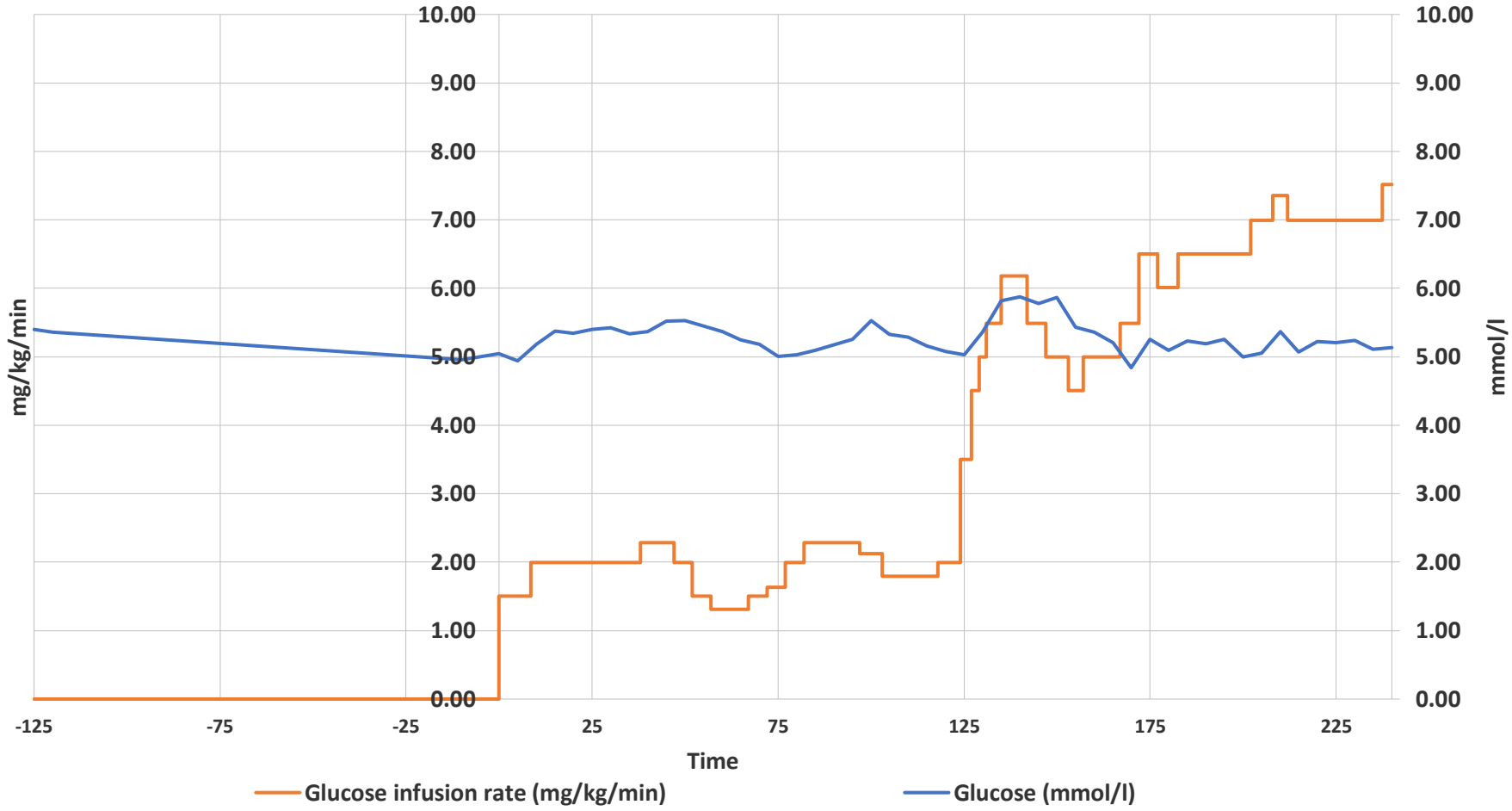


Figure 8: *A graphical representation of the plasma glucose (blue line) and dextrose infusion rate (orange line) during a single hyperinsulinaemic-euglycaemic clamp procedure.*

2.3.3 Magnetic resonance imaging

Magnetic resonance imaging (MRI) of the abdominal cavity was employed as a highly sensitive method to quantify visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and intrahepatic lipids (IHL). Participants were invited to attend a single visit at the clinical imaging department of Guy's Hospital London Bridge. A radiologist conducted the MRI scan and all prior safety checks. Participants were asked to lay in a supine position with body coils fixed in place. A 1.5 T Siemens Aera scanner was used to scan the abdominal cavity. Multiple contiguous axial T1-weighted gradient-echo images with a slice thickness of 3mm were obtained. From these images, the Dixon sequence was used to derive corresponding fat and water images for analysis by a blinded analyst using the open-source image analysis software HOROS V 1.1.7 (www.horosproject.org; accessed 21/10/2017).

Using the fat image from the L4/5 lumbar anatomical position, which aligns with the umbilicus, VAT and SAT area was highlighted to quantify VAT and SAT cm^2. To quantify IHL %, fat and water images at two abdominal positions, approximately 30mm apart encompassing the superior and inferior surface of the liver were analysed. Four identical circular regions of interest were positioned within the liver tissue in both pairs of images. The circles included the posterior, anterior, medial and lateral sections of the liver and aimed to cover as large an area as possible whilst avoiding blood vessels, ducts and fascia, thus the regions of interest were different for each participant. Within each area, the hepatic fat fraction (%) was calculated using the formula: (F/(F+W))*100, where F is the pixel signal intensity of the fat image and W is the pixel signal intensity of the water image. The mean hepatic fat fraction for all eight regions of interest was used as a measure of IHL% (334).

2.5 Sample laboratory analyses and materials

2.5.1 Automated assays

Plasma glucose concentration during the screening visit and the hyperinsulinaemic-euglycaemic clamp was measured at the bedside on an automated glucose analyser following centrifugation at room temperature for 15 seconds (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Yellow Springs, OH, USA). This method utilises a biosensor containing an immobilised enzyme membrane which oxidises the glucose from the sample into gluconolactone and hydrogen peroxide. The hydrogen peroxide is then oxidised, and the resulting electron flow is linearly proportional to the hydrogen peroxide and therefore the glucose concentration. The inter and intra coefficients of variation were <2%. For all other metabolites, samples were collected in BD vacutainers, centrifuged at 4°C at 3000rpm for 10 minutes to extract the plasma, which was stored at -80° for analysis in batches.

For insulin concentration analysis, samples were collected in plain vacutainers and allowed to clot for 20 minutes before centrifugation to extract the serum. This serum was measured by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare, Camberley, UK). This required the sample to be incubated with two insulin specific monoclonal mouse antibodies; one was labelled with an acridinium ester, and the other was covalently coupled to a paramagnetic particle. This created a two-site sandwich immunoassay with insulin being the protein of interest. Following an incubation period, a magnetic field was applied which held the insulin sandwich structure together in a solid phase on the sample reaction cuvette. Any components not bound to the magnetic field remained in the liquid phase and were removed. The cuvette was then washed with deionised water before a hydrogen peroxide reagent was added to begin the light emission reaction with the acridinium ester. The light

emission intensity was measured on a luminometer as relative light units which has a directly proportional relationship to the insulin concentration given in international units per litre (mIU/L). The intra-assay coefficient of variation was between 3.2 – 4.6% and the inter-assay coefficient of variation was between 2.6 – 5.9%.

Measurement of the screening blood samples (for triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol) was conducted in the central laboratory at King's College Hospital from SST vacutainers. For HbA1c, samples were collected in EDTA vacutainers and measured by boronate affinity and high-performance liquid chromatography (Premier Hb9210 analyser, Trinity Biotech, Jamestown, NY, USA). Glycated haemoglobins have a glucose moiety bound by a ketoamine bond which creates a diol group. Bonorate affinity chromatography involves the binding of a bonorate to this diol group and the glycated haemoglobin is quantified. The intra-assay coefficient of variation was between 0.72 – 1.26% and the inter-assay coefficient of variation was between 1.28 – 1.62%.

2.5.2 Isotope enrichment sample procedure

Whole blood samples were collected in fluoride and lithium heparin vacutainers for glucose and glycerol, respectively.

For assessment of glucose isotope enrichment, plasma samples were subjected to a two-step derivatisation process to create a penta-O-trimethylsilyl-D-glucose-O-methoxime derivative of natural and [6,6-$^{2}H_{2}$]-glucose. The resulting derivatives undergo electron impact ionisation, creating molecular fragments which can be identified and quantified using a combined gas chromatograph-mass spectrometer (GCMS) (Agilent GCMS 5975C MSD, Agilent Technologies, Wokingham, UK). The benefit of using a derivative is the ability to distinguish glucose from other monosaccharides in the plasma

matrix (335). The protocol for this method has been published (336) however in brief, the process was initiated with the centrifugation of 50µl of plasma and 500µl of ethyl alcohol which deproteinises the sample. The supernatant was then transferred to a dram vial where it was left to dry under oxygen-free nitrogen at 50°C for 20minutes in a sample concentrator (Techni, Philip Harris). For step 1 of the derivatisation process, 100µl of methylhydroxamine hydrochloride made up in pyridine 2% (w/v) was added to the dried residue and heated at 90°C for 2 hours. After being left to cool, step 2 of the derivatisation process was conducted by adding 50µl of N, O-bis(Trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane (BSTFA) to the residue which was then heated at 120°C for 15minutes. The derivatising agent was removed by leaving the solution to cool and dry under oxygen-free nitrogen at room temperature; the final product was reconstituted in 500µl of decane solvent for analysis in the GCMS. The derivatised solution was inserted into the gas chromatograph where it underwent electron ionisation using an ionisation energy of 70eV to induce ionised derivate fragments which are volatile and therefore detectable (335, 337, 338). These ions were accelerated then separated within the GCMS by electromagnetic deflection based on their charge to mass ratio (m/z) (160), the fragments were detected and quantified based on the fragment abundance. The electron impact ionisation mass spectra were obtained from m/z 650 to 50. Selected ion monitoring was used to measure the peak area under the curve for ions with a m/z of 319 and 321, representing natural glucose derivative and [6,6-$^{2}H_2$]-glucose derivative, respectively. The peak areas were calculated and expressed as the tracer (m/z 321 [6,6-$^{2}H_2$]-glucose)-to -tracee (m/z 319 glucose) ratio to quantify isotopic enrichment at each time point (335, 336).

For assessment of glycerol isotope enrichment, glycerol was first extracted from the plasma matrix before being subjected to a single derivatisation step and analysis by

GCMS. The protocol to assess glycerol enrichment has been published (161); however, the process was slightly modified. In brief, 500µl of plasma was deproteinised by centrifugation before being combined with 1000µl 3.5% (w/v) 5-sulphosalicylic acid solution to remove the fatty acids. Chromatography columns were set up to remove amino acids, lactate, pyruvate and other plasma components with either a positive or negative charge. Neutral plasma components, including glycerol, passed through the columns and the resulting eluate was collected and placed in a freeze drier overnight. For derivatisation, the dried residue was combined with 100µl of pyridine and 100µl N-methyl-N-[tert-butlydimethylsilyl]-trifluroacetamide and heated at 70°C for 4 hours. The sample was then left to stand at room temperature for 24 hours before the derivatising agent was removed under oxygen-free nitrogen. The final product was reconstituted in 50µl of decane solvent for analysis in the GCMS. Similar to the glucose enrichment analysis, the derivatised solution underwent gas chromatography and mass spectrometry. However, the selected ion monitoring mode used to detect and measure the peak area under the curve for fragment ions was m/z of 377 and 382, representing the natural glycerol derivative and [$^{2}H_{5}$]-glycerol derivative, respectively. The peak areas were calculated and expressed as the tracer (m/z 382 [$^{2}H_{5}$]-glycerol)-to -tracee (m/z 377 glycerol) ratio to quantify isotopic enrichment at each time point (161).

2.6 Calculations

2.6.1 Whole-body insulin sensitivity

Whole-body insulin sensitivity was expressed using the M value, which was calculated using the mean glucose (dextrose) infusion rate during the final 30 minutes of the high dose insulin infusion. At this point, a steady-state equilibrium was presumed to be present whereby the rate of glucose disposal equals the rate of glucose infusion. This

average glucose infusion rate is corrected for deviations in the plasma glucose pool. These adjustments were made every 10 minutes during the steady-state and were not a result of changes in glucose disposal, but rather too much or too little glucose infusion termed the 'space correction' (156). The space correction incorporates the change in glucose between two time points, the time between those timepoints and the total glucose volume distribution (assumed to be 19% of body weight) (156). The equation for the M value at each 10-minute interval is corrected for by this 'space correction' and the equation is as follows (the space correction is subtracted from the glucose infusion rate):

$$M\ (mg\ kg\ min^{-1}) = glucose\ infusion\ rate - \ (\frac{change\ in\ glucose * 10 * (0.19 * body\ weight)}{10 * body\ weight})$$

The results were averaged to provide the final M value (total glucose disposal). For comparisons between groups, total glucose disposal must be normalised for measurements of metabolic body size to account for the metabolic mass, which uses insulin during a clamp procedure (33, 84, 104). In this study glucose disposal was expressed as a function of body weight (mg/kg min^{-1}), body surface area (BSA; mg/m^2 BSA min^{-1}) and free fat mass (FFM; mg/kg FFM min^{-1}), where the data were available. Studies have shown that normalising for kg body weight, underestimate insulin sensitivity in obese participants due to obese participants having a larger proportion of tissue which is less responsive to insulin and thus FFM is the ideal normalisation to reflect metabolic mass which is sensitive to insulin (84, 104, 339). Whole-body insulin sensitivity was also measured as M/I ; (mg/m^2 BSA min^{-1})/(pmol/l) and (mg/kg FFM min^{-1})/(pmol/l), which is the total glucose disposal rate divided by insulin concentration during the final 30 minutes of the high dose insulin infusion. This expresses glucose disposal in relation to the prevailing insulin concentration, as discussed in chapter 1 (section 1.6) (89, 104, 156).

2.6.2 Tissue-specific insulin sensitivity

The isotopic tracer dilution technique can be used to quantify the appearance and disposal of glucose across body compartments, enabling a more accurate assessment of glucose production and disposal (105, 159). It can also be used to quantify the appearance of glycerol across body compartments, enabling a measure of total lipolysis (143, 144, 160, 161).

In its simplest form, during the basal state, the isotopic tracer dilution technique assumes a single compartment model for glucose and glycerol metabolism. Glucose and glycerol entry and removal from the compartment are at a steady-state equilibrium such that, the rate of appearance (Ra) equals the rate of disappearance (Rd). The technique requires a continuous infusion of a labelled isotopic tracer, which instantaneously and uniformly distributes throughout the pool until it reaches an equilibrium with the tracee. The Ra of any new glucose or glycerol (tracee) entering the system will be unlabelled, thus diluting the tracer concentration. This dilution provides a quantification for glucose or glycerol Ra.

$$\mathrm{Ra} = \frac{\mathrm{Tracer\ infusion}}{\mathrm{tracer - to - tracee - ratio}}$$

In humans, glucose is extracted by multiple compartments before entering the glucose pool from which it is sampled from. However during the basal state, the rate of glucose appearance equals the glucose disappearance creating a steady state. Insulin disrupts this steady-state therefore, the simplified single-compartment model cannot be used to assess glucose flux during the hyperinsulinaemic-euglycaemic clamp (159).

2.6.2.1 Peripheral and hepatic insulin sensitivity

To quantify the insulin-mediated increase in peripheral glucose disposal (peripheral insulin sensitivity) and the suppression of endogenous glucose production (primarily from the liver which reflects hepatic insulin sensitivity) during the clamp, modified versions of the single-compartment model equation have been employed (340). The modified equations provide quantification for the glucose Rd and Ra during the clamp steady states (the final 30 minutes of each insulin dose). The modifications to the equations account for the changes to steady-state metabolism which occur as a response to the insulin infusion.

Similar to the single-compartment model, the modified equations also require a quantification of the glucose isotope tracer. However, in addition to the continuous isotopic tracer infusion, the modified equations for non-steady state equations also account for the variable glucose infusion and the [6,6-$^{2}H_{2}$]-glucose tracer which has been mixed with the variable glucose infusion(333).

As discussed, the single-compartment model equation used at baseline requires the basal/steady-state tracer to tracee ratio (TTR). The tracer infusion is thought to reach an equilibrium during the final 30 minutes of the basal phase (after 90 minutes of infusion), such that the tracer and tracee flux is equal. A single value is used as the TTR during the basal/steady-state as the model assumes that the TTR is equal during the final 30 minutes of the basal state infusion. The TTR is also required to assess glucose dynamics during the clamp and is incorporated into the modified equations. However, in comparison to the basal state where the TTR is assumed to be steady throughout the basal state, the hyperinsulinaemic-euglycaemic clamp changes the TTR. During the clamp, compared to the basal TTR, the TTR rises as insulin stimulates glucose disposal and prevents endogenous glucose production. After 90 minutes, a new TTR equilibrium

(where the tracer and tracee flux are equal) is achieved and maintained throughout the procedure using the continuous isotope infusion and by enriching the variable glucose infusion with [6,6-$^{2}H_{2}$]-glucose. The average TTR and the change in TTR over time is incorporated into the modified equations to reflect this action. Optimal segments analysis (341) was used to smooth the TTR slope during the clamp to improve the fit with the mathematical equations.

The steady-state single-compartment model also assumes that the glucose concentration during the basal state does not change and is equal during the 30 minutes of the basal infusion. In comparison, the non-steady state modified equations are adapted to incorporate the changes in the glucose pool that occur during the clamp over time. Similar to the TTRs, optimal segments analysis (341) was used to smooth the glucose slope during the clamp to improve the fit with the mathematical equations. The model assumes that the total glucose volume distribution is 22% of the participant body weight; therefore, the change in glucose is adjusted to reflect the glucose volume distribution.

Finally, unlike the steady-state single-compartment model, the modified equations better acknowledge the complexity of glucose dynamics which are likely to be misrepresented in a single compartment model. The non-steady state equations account for the multiple compartment nature of glucose flux. The non-steady state equations introduce a coefficient which reflects the proportion of the glucose pool which is rapidly equilibrating compared to a slower equilibrating pool. This is more reflective of glucose dynamics. The model assumes that the readily mixable compartment (effective fraction) constitutes 65% of the total glucose pool (333, 340). The participants' body weight was multiplied by the effective fraction to provide the readily equilibrating glucose pool fraction. Whilst this coefficient is an improvement on the single-compartment model, it is still described to oversimplify the multicompartment nature of glucose flux and

maintaining a constant TTR, particularly in cases where glucose turnover is high after (e.g. after an insulin infusion), is important to prevent the oversimplification from producing invalid results (342).

Overall the non-steady state equations calculated at 10-minute intervals during the final 30 minutes of the basal, low and high dose insulin state are as follows:

$$\text{Total Glucose Ra} = \frac{D_2 \text{ glucose from the continuous infusion (mg/min)} + D_2 \text{ glucose from the various glucose infusion (mg/min)} - (\text{readily equilibrating glucose pool fraction} * \text{average glucose between time points (in mg/l)} * \text{change in TTR over time}}{\text{Average TTR}}$$

$$\text{Glucose Rd} = \text{Total Glucose Ra (mg/min)} - (\text{readily equilibrating glucose pool fraction} * \text{change in glucose over change in time}) + \text{variable glucose infusion (mg/min)}$$

The values are averaged to give the Ra and Rd during the final 30 minutes of the basal, low and high dose insulin infusion. During the basal state, the non-steady state modified equations provide the same result as the one-compartment model (section 2.6.2) because there is no exogenous glucose infusion and the TTR and glucose are modelled to be equal during the final 30 minutes of the basal infusion therefore, the change over time is 0.

The model provides measurements for the glucose Ra and Rd at baseline and during the clamp. This represents endogenous glucose production and peripheral glucose utilisation, respectively. A number of indices and measures which use these measurements to express insulin sensitivity are presented in this thesis:

- Peripheral insulin sensitivity was determined as the percentage increase in the rate of glucose disappearance (glucose Rd) from basal to the final 30 minutes of the high dose clamp (224, 239, 343).
- The peripheral insulin sensitivity index (PISI) was also calculated as the rate of glucose disappearance (glucose Rd) divided by the mean plasma insulin concentration during the final 30 minutes of the high dose clamp (153).
- Endogenous glucose production (glucose Ra) was calculated by subtracting the exogenous glucose infusion rate from total glucose Ra. Hepatic insulin sensitivity was measured as the percentage suppression of endogenous glucose production (glucose Ra) from basal to the final 30 minutes of the low dose insulin infusion of the clamp (163, 344)
- The hepatic insulin sensitivity index was also assessed during the basal and low dose insulin infusion of the clamp. This was calculated as the reciprocal of the product of endogenous glucose production (glucose Ra) and mean plasma insulin (153, 203, 204, 344).

2.6.2.2 Adipose tissue insulin sensitivity

A similar modelling methodology was employed to quantify the insulin-mediated suppression of total lipolysis during the clamp. Lipolysis involves the hydrolysis of triglycerides into glycerol and fatty acids, the former of which can be measured accurately (144). Whole-body/total lipolysis is primarily contributed by adipose tissue lipolysis, particularly during the basal state; therefore, changes in total lipolysis during a clamp reflect adipose tissue insulin sensitivity (143, 160).

Similar to basal glucose Ra, basal glycerol Ra was quantified using a single-compartment model. This required the quantification of the basal [$^{2}H_{5}$]-glycerol infusion

and basal TTR. The basal assessment of glycerol Ra also assumed the TTR and glycerol concentration was equal during the final 30 minutes of the basal infusion period.

During the non-steady state, unlike the glucose Ra equation, the equation to assess glycerol Ra was not modified to incorporate a readily mixable pool. The equation, therefore, assumed a single compartment model for glycerol flux. Also, for the non-steady state equation used to assess glycerol Ra, there was no exogenous glycerol applied during the clamp (outside of the isotope tracer) therefore, no additional modifications required. However, in line the equation used to assess glucose Ra during the clamp, the glycerol Ra equation was adjusted to incorporate the change in glycerol TTR equilibrium and changes in plasma glycerol concentration. The glycerol TTRs and glycerol concentrations were also smoothed with optimal segments analysis (341) to fit with the mathematical equations.

Modelling the glycerol data provides a direct measurement for the glycerol Ra at baseline and during the steady-state of the low dose insulin infusion of the clamp. This reflects whole-body/total lipolysis (primarily from adipose tissue) and has been used to express different measures of adipose tissue insulin sensitivity in this thesis:

- Adipose tissue insulin sensitivity was measured as the percentage suppression of whole-body/total lipolysis (glycerol Ra) from basal to the final 30 minutes of the low dose clamp (163).
- Adipose tissue insulin sensitivity was also estimated using the adipose tissue insulin sensitivity index (ATIS) at baseline and during the steady state of the low dose insulin infusion. ATIS is the reciprocal of the product of basal whole-body lipolysis (glycerol Ra) and mean basal plasma insulin (153).

2.7 Statistical analyses

Variables were assessed for normality using the Shapiro-Wilks test and histograms. Where data produced a normal distribution, summary statistics are presented as mean (SD). Data which required transformation (log 10) for normality, are presented as geometric mean (95% CI). Data which remained nonparametric, are presented as median (lower-upper IQR). Where appropriate, either two-way analysis of variance (ANOVA), a Student's T-test or Mann-Whitney U test was conducted to assess ethnic differences. In cases where the homogeneity of variance was not equal (assessed using the Levene's test) and therefore one of the assumptions behind the parametric ANOVA test had been violated, a more stringent statistical significance cut-off has been employed at $p<0.01$ (345, 346) otherwise, statistical significance was defined as $p <0.05$. Linear regression analyses were employed to adjust the mean difference in insulin sensitivity measures between BAM and WEM for fat, these data are presented as the unadjusted and adjusted mean difference (95% CI) calculated as the mean for the WEM minus the mean for the BAM. Pearson's correlation analyses were used to assess the associations between insulin sensitivity measures and fat. To determine whether these associations were ethnically distinct, an interaction term (ethnicity* fat depot) was applied using the linear multiple regression analysis. In addition to assessing the ethnicity interaction, the Y-intercept of the associations were compared using a t-test based on the intercepts themselves and their standard errors (SE). The test statistic was: t= (difference in intercepts)/(SE(difference). Data analyses were performed using SPSS software, version 25 (IBM Analytics, Armonk, NY, USA).

2.7.1 Power calculation

The Soul-DeEP study was powered on the primary outcome which was to assess insulin secretory function between black west African and white European men. Power calculations are traditionally based on randomised control trials and previously published data (347) which were far less extensive when the Soul-DeEP study was initially designed. Data reported at the time comparing multiple glucose tolerant groups using an intravenous glucose tolerance test with minimal modelling analysis found that differences in insulin secretory function were large (>1 standard deviation) (348-350) . Therefore, a two-group comparison of 20 vs 20 participants was deemed sufficient to detect a 1 standard deviation difference with 90% power at a 2 sided significant of 5%. Studies which also assessed lipolysis and insulin sensitivity using isotopic tracers and the intravenous glucose tolerance test (292, 351, 352), were also factored into the power calculation and it was concluded that a two group comparison of 20 vs 20 participants would be sufficient to detect and differences.

A retrospective power calculation was conducted based on an observation study by DeLany *et al.* who assessed tissue specific insulin sensitivity in lean normal glucose tolerant African-American and Caucasian women using the hyperinsulinaemic-euglycaemic clamp (322). This data suggested that to detect a 1 standard deviation difference with 90% power and a 2-sided significant of 5%, 56 participants per group are required.

Chapter 3: Description of participants

Chapter highlights:

- Black and white men were recruited to be similar in BMI however, this occurred in the presence lower waist circumference, visceral and hepatic fat in black men.
- Plasma triglycerides were lower and glycated hemoglobin was slightly higher in black men which are typical characteristics when comparing black and white populations.

3.1 Chapter introduction

There are multiple factors that influence insulin sensitivity which can be explained, in part, by evaluating the characteristics of the participants assessed. Insulin resistance associates significantly with obesity and adiposity thus, understanding participant characteristics ensures that conclusions drawn are based on those characteristics.

3.2 Aim

This chapter aims to describe the characteristics of the Soul-DeEP study participants who underwent a two-step hyperinsulinaemic-euglycaemic clamp.

3.3 Methods

3.3.1 Participants and study design

As detailed in chapter 2 (section 2.1 and 2.2), participants were recruited from the general population using various advertisements and through primary care by members of the research team. To confirm normal glucose tolerance (NGT) or impaired glucose tolerance (IGT), participants with no history of type 2 diabetes (T2D) underwent a 75g oral glucose tolerance test. Patients with T2D had been diagnosed by their physician within 5 years. Ethnicity was self-declared, and participants were categorised as either black west African or white European men (BAM or WEM, respectively). In addition to ethnicity and glucose tolerance, weight, height, waist circumference, blood pressure, glycated haemoglobin (HbA1c), plasma glucose, cholesterol and triglycerides were assessed at a screening visit. Finally, kidney and liver function tests were evaluated for eligibility. All participants provided informed consent prior to any metabolic assessments.

3.3.2 Statistical analyses

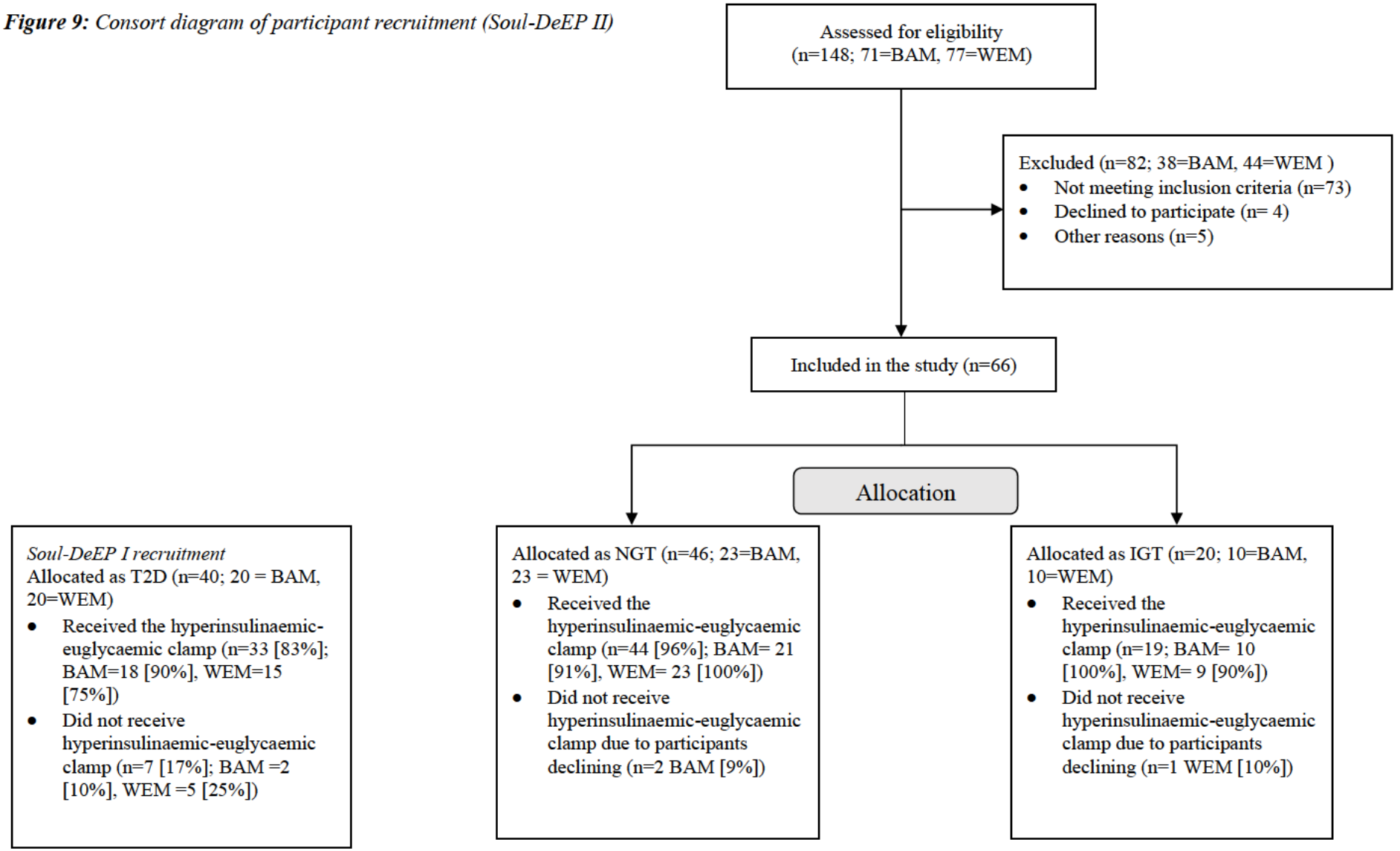
Each characteristic was assessed for normality using the Shapiro-Wilks test and histograms. Parametric data are presented as mean (SD); however, data which required transformation (log 10) for normality are presented as geometric mean (95% CI). Data which were nonparametric, and remained so after log 10 transformation, are presented as median (IQR). The main effect of ethnicity and glucose tolerance on each characteristic was assessed using a two-way analysis of variance (ANOVA), which assumes data are parametric. In cases where data were non-parametric, the ethnicity outcome of the two-way ANOVA was confirmed by the non-parametric Mann-Whitney U test in each glucose tolerance group. To assess the outcome of the ANOVA, the interaction statistic was interpreted before interpreting the main effect of ethnicity and glucose tolerance. To comply with statistical test assumptions of an ANOVA, in cases where the variance of the characteristic was unequal among groups (assessed using the Levene's statistic), a more stringent statistical significance cut-off has been employed at $p<0.01$. Otherwise, statistical significance was defined as $p<0.05$. Data analyses were performed using SPSS software, version 25 (IBM Analytics, Armonk, NY, USA).

3.4 Results

3.4.1 Participant recruitment

A consort diagram detailing non-diabetic participant recruitment for Soul-DeEP II, adapted to include the participants from Soul-DeEP I, is shown in figure 9. Within Soul-DeEP II, from April 2016 to January 2019, 148 individuals volunteered to take part in the study and were screened for eligibility. Of these, 31% were defined as NGT, 14% were defined as IGT and 55% were excluded for either not meeting the inclusion criteria, declining to participate or were found to be NGT after completion of recruitment into that category. Of the BAM assessed for eligibility in Soul-DeEP II, 32% (n=23) were allocated as NGT and 14% (n=10) were allocated as IGT. In comparison, 30% (n=23) of WEM were allocated as NGT and 13% (n=10) were allocated as IGT.

Figure 9: *Consort diagram of participant recruitment (Soul-DeEP II)*



3.4.2 Characteristics of black west African and white European participants

The characteristics of the Soul-DeEP participants are shown in table 2. Forty-nine BAM (21 NGT, 10 IGT,18 T2D) and 47 WEM (23 NGT, 9 IGT, 15 T2D) underwent a two-step hyperinsulinaemic-euglycaemic clamp to assess insulin sensitivity. The men were aged between 18-65 with an overall average age of 44. Eighty% (n=77) of participants were overweight/obese, as defined by a BMI of ≥25 kg/m^2, and this included almost all IGT and T2D participants. The remaining 20% (n=19) of lean participants were NGT, although 1 lean participant had IGT.

Concerning ethnicity, there was a non-significant trend towards BAM being younger than WEM, and this finding was based on a more stringent statistical significance cut-off to account for unequal variances between groups (see section 3.3.2). The lower age found in BAM compared to WEM was only statistically significant in men with IGT (p=0.02), the ethnic difference in age in men with NGT or T2D was not significant (p=0.18 and 0.51, respectively). The ethnic groups were similar in body weight and height and, as intended, the ethnic groups were similar in BMI within each glucose tolerance group; all averages fell within the overweight/obese BMI category. There were also no ethnic differences in body fat percentage or fat free mass, where the data were available for men with NGT or IGT. A trend towards lower waist circumference in BAM compared to WEM did not reach statistical significance but was consistent in each glucose tolerance group, there was an overall mean difference of 4.07cm when pooling all participants. Visceral adipose tissue (VAT) and intrahepatic lipid (IHL) content were also significantly lower in BAM compared to WEM. This finding was based on a more stringent statistical significance cut-off to account for unequal variances between groups (see section 3.3.2).The significant ethnic difference in VAT was significant within each glucose tolerance group (NGT p=0.02; IGT p<0.01;T2D p=0.02) and the ethnic difference in IHL

was statistically significant in participants with IGT (p=0.02) tended towards statistical significance in participants with T2D (p=0.07) and was not significant between participants with NGT (p=0.33). There were no ethnic differences in fasting plasma glucose or 2-hour glucose during the oral glucose tolerance test in participants without T2D, although there was a trend towards higher glycated haemoglobin (HbA1c) in BAM compared to WEM. In participants with T2D, there were no ethnic differences in diabetes duration or the proportion of those on metformin. There was a trend towards lower total cholesterol and significantly lower triglycerides in BAM compared to WEM which was particularly present between men with NGT (p<0.01) but not between men with IGT (0.29) or T2D (0.09). There were no ethnic differences in low-density lipoproteins cholesterol (LDL) or high-density lipoprotein cholesterol (HDL).

For each characteristic, there was no statistically significant interaction between ethnicity and glucose tolerance and the interaction statistic has been omitted from table 2. The main effect of glucose tolerance was statistically significant for all characteristics (p<0.05) excluding height, fat free mass, total cholesterol and HDL cholesterol. The post hoc test for the characteristics which did show a significant effect of glucose tolerance are as follows. Age, BMI, waist circumference, VAT, triglycerides and blood pressure were significantly different between participants with NGT and IGT or T2D however there was no significant difference between participants with IGT and T2D. Body weight and IHL was significantly different between participants with NGT and IGT (p<0.01) however, there were no significant differences between any of the other glucose tolerance groups. SAT was significantly different between participants with NGT and T2D (p=0.03) however there were no significant differences between any other groups. Fasting plasma glucose and HbA1c were statistically different between all glucose tolerance

groups. Finally, LDL was not significantly different between participants with NGT or IGT but T2D and IGT or NGT showed a significant difference.

Table 2: *Characteristics of black west African and white European men who underwent a hyperinsulinaemic-euglycaemic clamp*

	NGT		IGT		T2D			
Characteristic	**BAM 21**	**WEM 23**	**BAM 10**	**WEM 9**	**BAM 18**	**WEM 15**	P_1 **Ethnicity**	P_2 **Glucose tolerance**
Age (years) [a]	25.0 (22.0 – 40.0)	29.0 (25.0 – 53.0)	45.5 (41.0 – 49.8)	57.0 (47.0 – 63.5)	54.0 (48.0 – 60.3)	59.0 (53.0 – 60.0)	0.02	<0.01
BMI (kg/m^2) [b]	26.61 (25.08, 28.22)	26.18 (24.37, 28.13)	30.91 (28.47, 33.55)	30.59 (27.72, 33.76)	29.40 (28.08, 30.78)	29.97 (28.47, 31.55)	0.93	<0.01
Body weight (kg)	84.9 (14.9)	86.6 (16.5)	97.7 (14.7)	97.2 (14.6)	90.9 (9.3)	94.2 (11.7)	0.62	<0.01
Height (cm)	177.5 (7.8)	180.3 (5.8)	177.0 (6.0)	177.4 (5.0)	175.6 (7.6)	176.8 (5.8)	0.31	0.20
Body fat (%)	20.67 (6.21)	20.85 (6.81)	25.73 (4.98)	25.30 (6.09)	-	-	0.94	<0.01
Fat free mass (FFM) (kg) [a]	66.8 (62.0 – 69.3)	68.8 (61.3 – 71.9)	69.5 (64.8 – 78.9)	69.7 (65.9 – 76.1)	-	-	0.91	0.11
Waist circumference (cm) [b]	87.5 (83.4, 91.8)	92.8 (87.1, 99.0)	102.1 (94.4, 110.3)	106.6 (98.6, 115.1)	103.3 (99.2, 107.4)	107.2 (102.4, 112.2)	0.05	<0.01

Continued

Table 2: *Characteristics of black west African and white European men who underwent a hyperinsulinaemic-euglycaemic clamp*

	NGT		IGT		T2D			
Characteristic	**BAM 19**	**WEM 23**	**BAM 10**	**WEM 9**	**BAM 18**	**WEM 15**	P_1 Ethnicity	P_2 Glucose tolerance
MRI and MRS body composition measures								
Subcutaneous adipose tissue (SAT) L4/5 [ac]	188.7 (91.6 – 241.0)	220.7 (126.7 – 262.1)	212.9 (187.2 – 397.0)	270.3 (243.1 – 299.9)	279.0 (215.8 – 347.7)	247.8 (228.5 – 318.9)	0.79	0.03
Visceral adipose tissue (VAT) L4/5 [bc]	45.29 (33.34, 61.51)	78.96 (55.44, 112.47)	86.49 (62.71, 119.29)	174.49 (114.66, 265.53)	125.17 (103.50, 151.36)	175.89 (140.23, 220.62)	<0.01	<0.01
Intrahepatic lipids (IHL) (%) [ad]	3.45 (2.96 – 4.47)	3.68 (3.07 – 6.41)	3.55 (3.30 – 6.53)	9.90 (4.85 – 20.75)	3.66 (2.87 – 6.83)	5.20 (4.29 – 10.17)	<0.01	0.01

Continued

Table 2: *Characteristics of black west African and white European men who underwent a hyperinsulinaemic-euglycaemic clamp*

	NGT		IGT		T2D			
Characteristic	**BAM 21**	**WEM 23**	**BAM 10**	**WEM 8**	**BAM 18**	**WEM 15**	P_1 **Ethnicity**	P_2 **Glucose tolerance**
Glycaemic markers								
Fasting glucose (mmol/l) [b]	5.10 (4.90, 5.31)	5.18 (5.02, 5.35)	5.59 (5.28, 5.91)	5.73 (5.17, 6.36)	6.60 (6.14, 7.08)	6.68 (5.97, 7.47)	0.50	<0.01
2-hour glucose (mmol/l)	5.28 (1.13)	5.09 (1.26)	9.17 (1.15)	9.07 (1.26)	-	-	0.67	<0.01
HbA_{1c} (mmol/mol)	36.90 (5.51)	35.87 (2.88)	43.50 (4.33)	40.00 (2.40)	50.44 (7.49)	48.60 (7.82)	0.08	<0.01
HbA_{1c} (%)	5.53 (0.50)	5.44 (0.24)	6.13 (0.39)	5.80 (0.24)	6.76 (0.66)	6.60 (0.72)	0.08	<0.01
Duration of diabetes (years) [a]	-	-	-	-	3.0 (2.5, 3.6)	3.0 (2.0, 4.0)	0.74	-
Treated with metformin (%)	-	-	-	-	78	53	0.16	-

Continued

Table 2: *Characteristics of black west African and white European men who underwent a hyperinsulinaemic-euglycaemic clamp*

Characteristic	NGT		IGT		T2D		P_1 Ethnicity	P_2 Glucose tolerance
	BAM 21	WEM 23	BAM 10	WEM 9	BAM 18	WEM 15		
Lipids								
Total cholesterol (mmol/l) [b]	4.26 (3.85, 4.73)	4.65 (4.23, 5.11)	4.43 (3.88, 5.06)	4.91 (4.38, 5.50)	4.12 (3.79, 4.48)	4.25 (3.89, 4.63)	0.08	0.14
LDL-cholesterol (mmol/l) [b]	2.62 (2.30, 3.00)	2.88 (2.54, 3.25)	2.85 (2.47, 3.30)	2.94 (2.43, 3.54)	2.31 (2.07, 2.58)	2.20 (1.88, 2.57)	0.69	<0.01
HDL-cholesterol (mmol/l) [a]	1.20 (1.15 – 1.40)	1.20 (1.10 – 1.40)	1.05 (0.88 – 1.35)	1.40 (0.90 – 1.55)	1.15 (0.88 – 1.40)	1.20 (1.00 – 1.40)	0.20	0.49
Triglycerides (mmol/l) [b]	0.67 (0.59, 0.77)	0.99 (0.81, 1.21)	1.03 (0.78, 1.36)	1.27 (0.89, 1.81)	1.20 (0.95, 1.52)	1.58 (1.26, 1.97)	<0.01	<0.01

Continued

Table 2: *Characteristics of black west African and white European men who underwent a hyperinsulinaemic-euglycaemic clamp*

Characteristic	NGT		IGT		T2D			
	BAM 21	WEM 23	BAM 10	WEM 9	BAM 18	WEM 15	P_1 Ethnicity	P_2 Glucose tolerance
Blood pressure								
Systolic BP (mmHg)	124.0 (11.9)	121.9 (9.1)	132.7 (10.0)	130.6 (14.3)	138.4 (13.6)	131.8 (13.9)	0.17	<0.01
Diastolic BP (mmHg)[b]	70.3 (65.5, 75.5)	70.7 (67.2, 74.3)	82.9 (78.5, 87.5)	79.2 (75.4, 83.3)	86.7 (84.2, 89.4)	82.4 (77.2, 87.9)	0.22	<0.01

Data expressed as mean (SD) for parametric data unless identified otherwise or as a percentage of individuals

P_1 : Main effect of ethnicity, P_2 : Main effect of glucose tolerance

[a]Median (interquartile range) for non-parametric data , [b]Geometric mean (95% CI) for log-transformed data , [c]Sample size: WEM T2D=14 , [d]Sample size: BAM T2D=16

3.5 Discussion

Data summarised in this chapter describes the characteristics of the participants who underwent a hyperinsulinaemic-euglycaemic clamp in the Soul-DeEP study. The analyses suggested that there was a trend towards lower age in black men compared to white men, particularly those with impaired glucose tolerance. Although there was an ethnic difference in age, the ethnic difference in age may not be a confounding factor which requires statistical adjustment when interpreting insulin sensitivity findings. In support of this, there is evidence to suggest that black communities are at greater risk of developing type 2 diabetes (T2D) at a younger age compared to white communities. Data from the UK has shown black African Caribbeans being diagnosed with T2D up to 10 years earlier compared to white Europeans (63, 67). Thus, the age difference in this analysis suggests that the participant sample is reflective of this high-risk population with early-onset dysglycaemia. In further support of the age difference not being a confounding factor in the assessment of insulin sensitivity, several studies have suggested that insulin sensitivity is not associated with age, including a study in black men and women authored by Goedecke *et al.* (353). Goedecke and colleagues assessed insulin sensitivity in 439 participants between the ages of 25 and 74 with normal glucose tolerance. Insulin sensitivity was estimated based on an oral glucose tolerance test derived index and fasting indices of insulin sensitivity. The results showed no significant association between insulin sensitivity and age in both men and women however, insulin sensitivity was associated with BMI (353). The data from Goedecke *et al.* and other research articles suggest that insulin sensitivity is not independently associated with age, rather insulin sensitivity is associated with age-related changes in body composition (259, 260, 354). To account for this, participants were recruited to be similar in BMI between ethnic groups within each glucose tolerance group. BMI is of importance due to the

consistent link between obesity and T2D (23, 24). The data here show that despite having similar BMIs, black men presented with lower waist circumference, ectopic fat, visceral fat, circulating lipids but slightly higher HbA1c compared to white European men. These ethnic differences have been reported elsewhere (247, 256, 279, 355-358) and support the suggestion that this sample of participants was representative of the general population. Data from this study shows no evidence of an ethnic difference in SAT which agrees with studies in adolescents and adults (359, 360). However, the literature on whether there is an ethnic difference in SAT is highly contradictory with other reports showing an ethnic difference (361). These inconsistences may be due to differences in the tools used to measure SAT.

All characteristics measured were affected by glucose tolerance, and this occurred independently of ethnicity. This finding was to be expected due to the progressive and multifactorial nature of type 2 diabetes (71) as well as the fact that the study design was centred around ethnicity and matching for this within glucose tolerance groups.

In addition to the participant characteristics, this chapter also describes the recruitment data from the Soul-DeEP study. The study aimed to recruit men with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and T2D. In particular, the recruitment of IGT is thought to be more difficult than recruiting individuals with NGT or T2D because there are limited clinical diagnostic features and they are not routinely sought in clinical practice.

To conclude, the participants assessed in this thesis were recruited as part of the Soul-DeEP study which was able to recruit black men and men with impaired glucose tolerance, both of which have traditionally been identified as a difficult to reach groups. The characteristic data suggests the cohort can be considered representative of the population from which the sample was drawn.

Chapter 4: A comparison of whole-body insulin sensitivity between black and white men

Chapter highlights:

- Whole-body insulin sensitivity was similar in both ethnic groups
    - Normalising whole-body insulin sensitivity for different expressions of metabolic size did not alter the ethnicity findings, BSA will be used in all other chapters going forward.
    - Normalising whole-body insulin sensitivity for insulin did not alter the ethnicity findings
- Similar whole-body insulin sensitivity occurred in the presence of lower VAT in BAM compared to WEM
- Adjusting whole-body insulin sensitivity for VAT resulted in lower whole-body insulin sensitivity in BAM. Lower VAT may protect BAM from being more insulin resistant.

Data presented in this chapter have been published:

Bello O, Mohandas C, Shojaee-Moradie F, Jackson N, Hakim O, Alberti KGMM, Peacock JL, Umpleby AM, Amiel SA, Goff LM. Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat. Diabetologia. 2019 May;62(5):835-844.

Bello O, Ladwa M, Hakim O, Marathe C, Shojaee-Moradie F, Charles-Edwards G, Peacock JL, Umpleby MA, Amiel S, Goff LM. Differences in the link between insulin sensitivity and ectopic fat in men of Black African and White European ethnicity. Eur J Endocrinol. 2019 Nov;182 (1):91-101

4.1 Introduction

Insulin sensitivity is a measure of responsiveness to insulin, a decline in such is termed insulin resistance and is an early defect in the pathophysiology of type 2 diabetes (T2D) (95, 157). Insulin is an integral driver of glucose homeostasis. It has been shown to 1) increase glucose disposal in peripheral tissues by promoting glucose uptake, storage and oxidation and 2) suppress endogenous glucose production in the liver (71, 362). The result of these actions is a reduction in circulating glucose. The term "whole-body insulin sensitivity" generally refers to the combined effects of insulin-mediated total glucose disposal and the suppression of endogenous glucose production. The hyperinsulinaemic-euglycaemic clamp has been termed the "gold standard" *in vivo* method to assess whole-body insulin sensitivity directly (156, 157). The procedure involves a peripheral insulin infusion which is thought to suppress endogenous glucose production. The glucose infusion required to maintain euglycemia during the hyperinsulinaemic procedure, M value, is a measure of whole-body insulin sensitivity to glucose metabolism. The M value is normalised by expressing the data per unit of metabolic body size, e.g. per kg body weight, to allow for comparison amongst different individuals (84, 104). Studies which have assessed whole-body insulin sensitivity in adults with T2D show insulin action to be at least 38% lower in comparison to those without T2D (75, 97).

Efforts to understand what influences insulin sensitivity to fall or insulin resistance to increase have implicated excess adiposity as a culprit. Various indirect and direct methods have consistently linked insulin resistance to adiposity, particularly central fat (182, 363). An increase in visceral adipose tissue (VAT), a highly lipolytic fat store, has been linked with whole-body insulin resistance (182, 186). Animal studies have provided evidence which supports the causal effect of VAT on insulin resistance (201).

The potential mechanisms which link VAT and insulin sensitivity have been discussed in chapter 1 (section 1.7.1).

Assessments of insulin sensitivity using surrogate indices or the intravenous glucose tolerance test with minimal model analysis have almost consistently shown increased insulin resistance in black compared to white participants, particularly in participants without T2D as reviewed by Kodama and colleagues (265). Their systematic review of 71 cohorts included 801 Africans-Americans and 2454 Caucasian participants who had either normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or T2D. Their analysis excluded studies who had mainly recruited obese participants and although the black and white participants were similar in BMI, insulin resistance was greater in African-Americans compared to Caucasian participants. In comparison, studies which have directly assessed and compared whole-body insulin sensitivity in black and white adults using the hyperinsulinaemic-euglycaemic clamp have produced inconsistent findings. The majority of studies have focused on obese non-diabetic women with the average BMI ranging from 32 - 26 kg/m^2. The sample sizes also ranged from 9 to 10 black women and 10 to 26 which women. These studies have reported up to 60% lower insulin sensitivity in black compared to white women (245, 251, 311). More recent data from the POP-ABC study which includes men and women across a range of BMIs have also assessed insulin sensitivity derived from the clamp however , participants were not matched for BMI such that the black participants were more obese which may confound the insulin sensitivity findings. Their data has also shown lower (or a trend towards lower) insulin sensitivity in black compared to white participants (319-321). A single study which matched participants for BMI (the average BMI of 29.0 kg/m^2) has provided evidence for 25% greater insulin sensitivity in non-diabetic black compared to white men (312). Whilst these clamp studies report an ethnic difference in whole-body insulin

sensitivity, there are also multiple reports of no ethnic difference in whole-body insulin sensitivity between black and white participants (307, 312-318). These studies contain participants with a range of BMIs and glucose tolerances. However, the black and white participants were not consistently matched for BMI such that black participants were heavier which may confound results considering the influence of adiposity, particularly central adiposity, on insulin sensitivity.

Overall, the inconsistencies in the ethnic findings from clamp studies assessing whole-body insulin sensitivity may reflect differences in participant populations such as sex, glucose tolerance, family history, and whether BMI was matched. The majority of studies have assessed obese women and participants without T2D. Some studies have not matched for BMI. The impact of not matching for BMI and whether findings from obese women can be extrapolated to men with a range of glucose tolerances and BMI statuses are unknown.

4.2 Aim

This chapter aims to determine whether there are ethnic differences in whole-body insulin sensitivity to glucose metabolism in black west African (BAM) and white European men (WEM) with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or early type 2 diabetes (T2D).

It could be hypothesised that BAM without T2D will have lower whole-body insulin sensitivity than WEM. In T2D, there will be no ethnic difference in whole-body insulin sensitivity.

4.3 Methods

Details for the study methods including the participant inclusion criteria and metabolic assessments can be found in chapter 2. In brief, black west African (BAM) and white European men (WEM) were categorised as normal glucose tolerant (NGT) or impaired glucose tolerant (IGT), based on their oral glucose tolerance test, or as having type 2 diabetes (T2D) if diagnosed within 5 years by their primary care practitioner.

Participants were invited to attend a two-step hyperinsulinaemic-euglycaemic clamp with a continuous insulin infusion at 10mU/m^2BSA/min and 40mU/m^2BSA/min for 2 hours each. During the insulin infusion, a steady-state equilibrium is achieved whereby the glucose infused to maintain euglycemia (5 mmol/l) equilibrates to the total glucose disposal from the circulation. The steady-state equilibrium is defined as the final 30 minutes of each insulin infusion; blood samples were drawn during steady states as well as the basal period for assessment of plasma glucose and insulin.

On a separate day, participants also attended a magnetic resonance imaging scan to assess visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) area at anatomical position L4/5. Intrahepatic lipid (IHL) was also assessed as the average hepatic fat fraction from 8 circular regions of interests from the superior and inferior surface of the liver.

4.3.1 Calculations

Whole-body insulin sensitivity was assessed using the M value, which computes the mean total glucose disposal during the final 30 minutes of the high dose insulin infusion. The M value was normalised per person by expressing glucose disposal per kg body weight (mg/kg min^{-1}), m^2 body surface area (BSA; mg/m^2 BSA min^{-1}) and per kg fat-free mass (FFM; mg/kg FFM min^{-1}) where the data were available. The latter two

have been suggested to be a more accurate assessment of insulin sensitivity which are less likely to underestimate insulin sensitivity in obese participants, which has been found with a kg body weight normalisation (84, 104, 339). To account for background insulin exposure, M value was divided by mean plasma insulin during the final 30 minutes of the clamp to generate M/I; (mg/m^2 BSA min^{-1})/(pmol/l) and (mg/kg FFM min^{-1})/(pmol/l) (104, 156).

4.3.2 Statistical analyses

Data for each dependent variable were assessed for normality using the Shapiro-Wilks test and histograms. Parametric data are presented as mean (SD), data which required transformation (log 10) are presented as geometric mean (95% CI) and data which remained non-parametric are presented as median (interquartile range). Mean glucose and insulin concentrations during the basal and steady-state periods were compared by an independent samples t-test or Mann-Whitney U test where data were parametric and non-parametric, respectively. The relationship between basal glucose and insulin concentrations were assessed in participants with and without T2D using a Persons' correlation co-efficient.

To assess the main effect of ethnicity and glucose tolerance, a two-way analysis of variance (ANOVA) was conducted. To assess the outcome of the ANOVA, the interaction statistic was interpreted before interpreting the main effect of ethnicity and glucose tolerance. Where the interaction statistic was non-significant, the main effect of ethnicity and glucose tolerance were independent of one another, thus interpreted in isolation. Glucose tolerance had more than 2 groups (NGT, IGT, T2D); therefore, where a significant main effect was found, the Tukey's post hoc tests were employed to determine where the differences lay. To control for the effect of ethnic differences in VAT

shown in chapter 3 (section 3.4.2), linear regression was employed to assess the effect of ethnicity on whole-body insulin sensitivity whilst adjusting for glucose tolerance, VAT and IHL. These data are reported as the unadjusted and adjusted mean difference and 95% CI (WEM minus BAM). Statistical significance was defined as $p<0.05$ and data analyses were performed using SPSS software, version 25 (IBM Analytics, Armonk, NY, USA).

4.4 Results

The characteristics of the participants have been described in chapter 3. Briefly, men were between the ages of 18 to 65 years, with an average BMI within the overweight and obese category. There were no ethnic differences in BMI status in each glucose tolerance group (p=0.93); however, BAM were younger compared to WEM, particularly those with impaired glucose tolerance (p=0.02). Despite no ethnic difference in BMI status, there was a non-significant trend towards lower waist circumference in BAM (p=0.05) and significantly lower visceral and hepatic fat in BAM compared to WEM (p<0.01 and p<0.01, respectively). Plasma triglycerides were significantly lower in BAM compared to WEM (P<0.01) and total cholesterol tended to be lower in BAM compared to WEM (p=0.08).

4.4.1 Glucose and insulin during the clamp procedure

Glucose and insulin concentrations during the clamp time course are shown in figure 10A for participants with NGT, figure 10B for participants with IGT and figure 10C for participants with T2D. Table 3 summarises of the average glucose and insulin concentrations during the basal and steady-state periods of the clamp. During each steady-state, there were no ethnic differences in mean glucose in any glucose tolerance group. The mean insulin concentration during the basal and low dose insulin infusion was no different between BAM or WEM in any glucose tolerance. During the high dose insulin infusion, there were no ethnic differences in insulin in participants with IGT or T2D. However, in participants with NGT, a trend towards higher mean insulin concentration in BAM compared to WEM was present, mean difference and 95% CI 53.1 (-1.5, 107.6) pmol/l, but did not reach statistical significance. When compared to the basal state, on average in NGT, the insulin concentration rose by 12.0 times in BAM and 12.1 times in

WEM which was not statistically different (p=0.96). Overall, there were no ethnic differences in the average increase in insulin during the low or high dose insulin infusion in any glucose tolerance group (p>0.26).

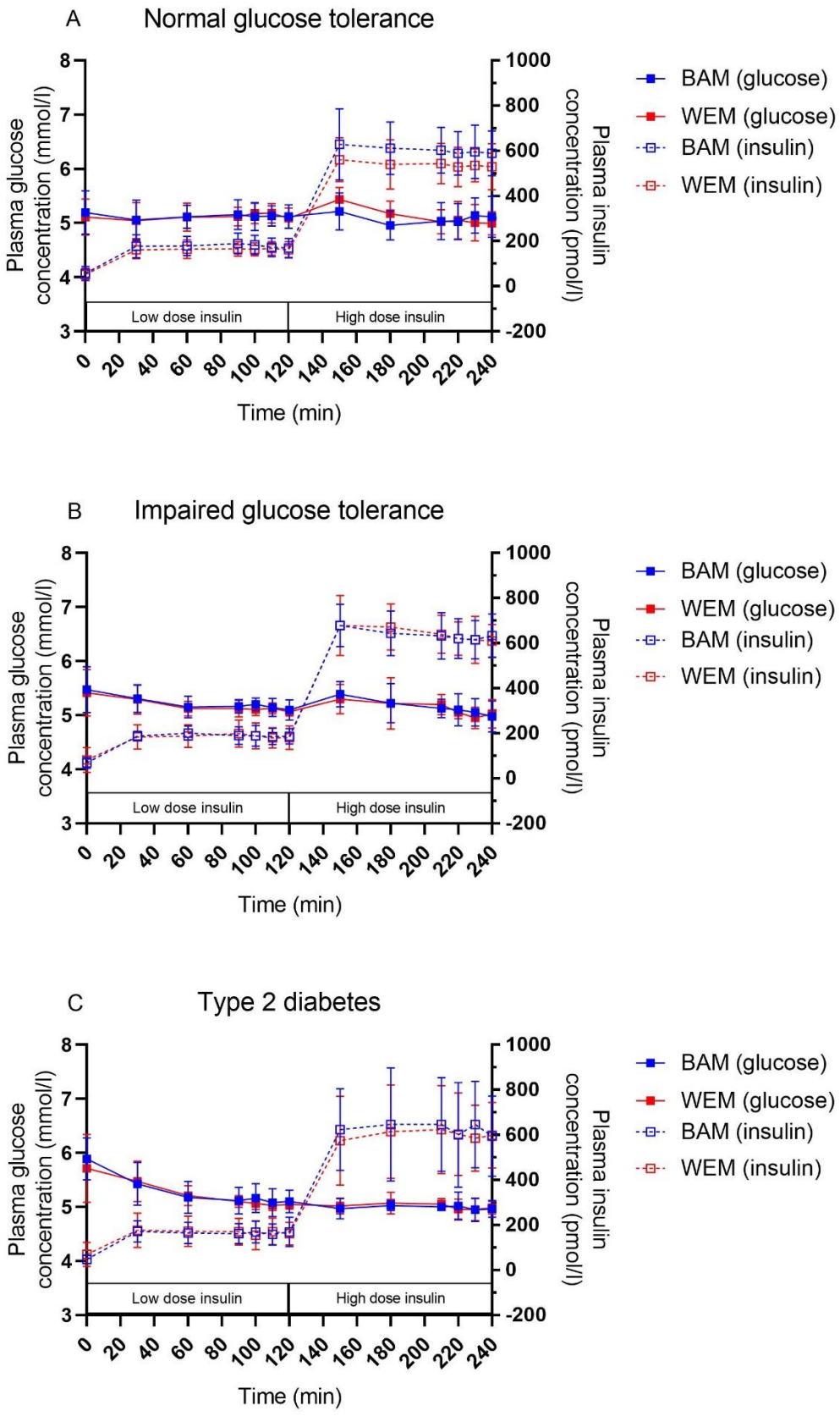


Figure 10: *Glucose and insulin concentrations during the clamp in black west African (BAM) and white European men (WEM) with A) normal glucose tolerance B) impaired glucose tolerance and C) type 2 diabetes. Data expressed as mean (SD) per time point.*

Table 3: *Glucose and insulin concentrations during the different phases of the hyperinsulinaemic-euglycaemic clamp*

	NGT			IGT			T2D		
Clamp phase	**BAM 21**	**WEM 23**	***P***	**BAM 10**	**WEM 9**	***P***	**BAM 18**	**WEM 15**	***P***
Insulin (pmol/l)									
Basal	51.7 (41.8, 64.0)[a]	47.2 (38.5, 58.0)[a]	0.53	68.8 (24.0)	81.6 (55.6)	0.54	44.5 (36.4, 54.4)[ad]	57.2 (39.2, 83.5)[ad]	0.19
Low dose insulin infusion	173.6 (157.3, 191.6)[ac]	161.8 (149.3, 175.3)[a]	0.25	186.8 (32.9)	186.1 (49.6)	0.97	164.3 (44.7)	164.3 (55.9)	1.00
High dose insulin infusion	588.1 (94.1)[c]	535.0 (82.9)	0.06	620.1 (80.1)	617.4 (73.6)	0.94	622.0 (181.5)	612.0 (166.8)	0.87
Glucose (mmol/l)									
Basal	5.19 (0.40)	5.11 (0.33)	0.45	5.47 (0.42)	5.42 (0.43)	0.77	5.89 (0.39)[d]	5.71 (0.63)[d]	0.38
Low dose insulin infusion	5.11 (5.04 – 5.21)[bc]	5.14 (5.07 – 5.22)[b]	0.47	5.15 (0.13)	5.11 (0.10)	0.41	5.03 (4.98 – 5.25)[b]	5.07 (5.00 – 5.10)[b]	0.80
High dose insulin infusion	5.09 (0.17)[c]	5.02 (0.22)	0.25	5.07 (0.17)	5.06 (0.08)	0.85	4.98 (0.14)	4.99 (0.09)	0.94

Data are expressed as mean (SD)
[a]Geometric mean (95% CI) , [b]Median (IQR), [c]Sample size: BAM=20 , [d]Sample size: BAM= 15, WEM=12

4.4.2 Insulin and glucose relationship

The association between basal glucose and log-transformed basal insulin was statistically significant in BAM (r=0.59, p<0.01) and WEM (r=0.47 P<0.01) without T2D. There was no statistically significant association between basal glucose and insulin in BAM (r=-0.33, p=0.18) or WEM (r=-0.16, p=0.57) with T2D. The data have been presented in figure 11 containing all participants.

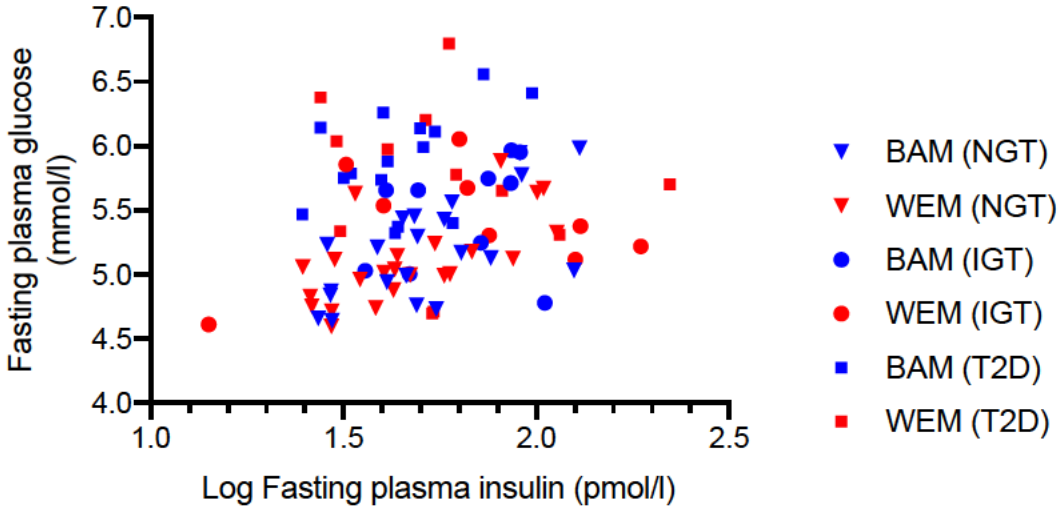


Figure 11: *The relationship between glucose and insulin in men with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2D).*

4.4.3 Whole-body insulin sensitivity

Whole-body insulin sensitivity, computed as the M value, expressed as kg body weight, BSA, kg FFM and as M/I, are presented in table 4 and BSA expressions are also shown in figure 12A. In each glucose tolerance group, there were no ethnic differences in whole-body insulin sensitivity in all expressions of the M value. When expressing M as a function of insulin (M/I), the main effect of ethnicity on whole-body insulin sensitivity remained non-significant (table 4, figure 12B).

There was a statistically significant main effect of glucose tolerance on all expressions of the M value ($p<0.01$). The post hoc analysis indicated that for all expressions of whole-body insulin sensitivity, participants with NGT had statistically higher insulin sensitivity than those with IGT or T2D ($p<0.01$). However, there was no statistical difference between whole-body insulin sensitivity in men with IGT and T2D ($p>0.92$).

Table 4: *Whole-body insulin sensitivity during the hyperinsulinaemic-euglycaemic clamp*

	NGT		IGT		T2D				
Glucose disposal rate measure	**BAM 20**	**WEM 23**	**BAM 10**	**WEM 9**	**BAM 18**	**WEM 15**	P_1 **Ethnicity**	P_2 **Glucose tolerance**	P_3 **Interaction**
M; mg/kg min^{-1}	7.72 (2.32)	7.71 (3.43)	4.50 (1.40)	4.36 (1.35)	4.52 (2.07)	4.00 (1.71)	0.66	<0.01	0.98
M; mg/m^2 BSA min^{-1}	315.4 (76.1)	309.6 (127.5)	197.0 (59.9)	188.2 (51.1)	191.0 (85.3)	172.6 (69.7)	0.57	<0.01	0.92
M/I; (mg/m^2 BSA min^{-1})/(pmol/l)	0.56 (0.20)	0.62 (0.31)	0.32 (0.11)	0.32 (0.11)	0.34 (0.19)	0.30 (0.11)	0.97	<0.01	0.56
M; mg/kg FFM min^{-1}	9.65 (2.32)	9.51 (3.86)	6.02 (1.77)	5.79 (1.59)	-	-	0.82	<0.01	0.95
M/I; (mg/kg FFM min^{-1})/(pmol/l)	17.1 $x10^{-3}$ ($5.9\ x10^{-3}$)	18.9 $x10^{-3}$ ($9.4\ x10^{-3}$)	9.84 $x10^{-3}$ ($3.29\ x10^{-3}$)	9.69 $x10^{-3}$ ($3.46\ x10^{-3}$)	-	-	0.66	<0.01	0.61

Data are expressed as mean (SD)

P_1 :Main effect of ethnicity, P_2 :Main effect of glucose tolerance, P_3 : Ethnicity and glucose tolerance interaction

Measures of whole-body insulin sensitivity in men with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2D) of black west African (BAM) or white European (WEM) ethnicity.

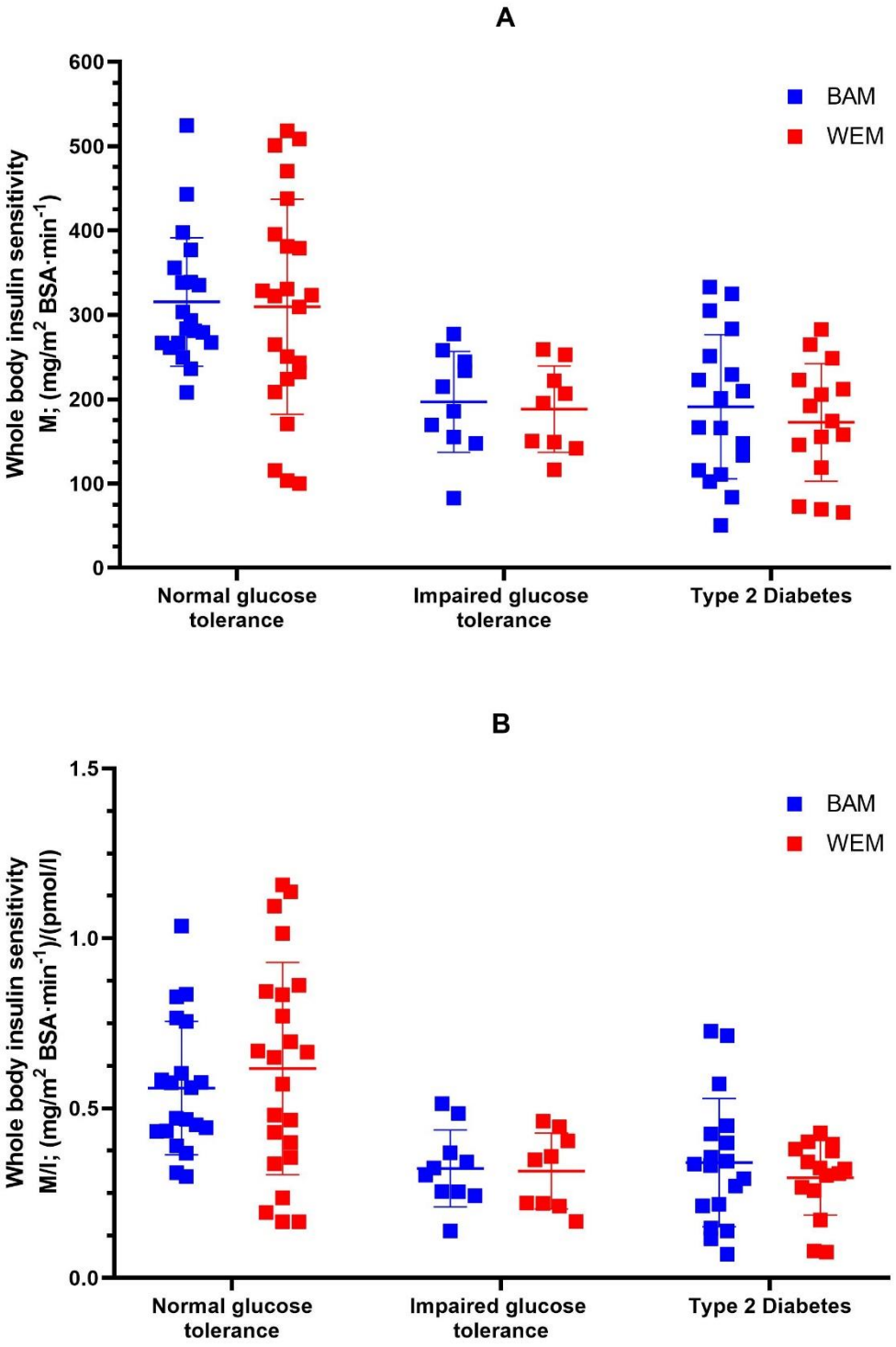


Figure 12: *Whole-body insulin sensitivity in black west African (BAM) and white European men (WEM) by glucose tolerance.*

Data are presented as mean (SD).
Whole-body insulin sensitivity, M value has been expressed as body surface area (BSA).

4.4.4 Whole-body insulin sensitivity adjustments for VAT and IHL

The ethnic and glucose tolerance comparison of VAT and IHL in chapter 3 (section 3.4.2) showed a statistically significant main effect of ethnicity and glucose tolerance. VAT and IHL was lower in BAM compared to WEM ($p<0.01$). VAT was also lower in NGT compared to IGT or T2D ($p<0.01$); however, there was no difference between IGT to T2D ($p=0.47$). IHL was lower in NGT compared to IGT ($p=0.04$); however, there was no difference between participants with NGT and T2D or participants with IGT and T2D ($p>0.14$)

The overall unadjusted mean difference and 95% CI of whole-body insulin sensitivity (M; mg/m^2 BSA min^{-1}) between BAM and WEM was non-significantly higher in BAM at -1.45 (-46.6, 43.7) mg/m^2 BSA min^{-1}. Adjusting whole-body insulin sensitivity (M; mg/m^2 BSA min^{-1}) for VAT resulted in significantly lower insulin sensitivity in BAM with an overall adjusted mean difference and 95% CI of 34.1 (3.09, 65.05) mg/m^2 BSA min^{-1}, $p=0.03$. Adjusting whole-body insulin sensitivity for IHL resulted in a trend towards lower insulin sensitivity in BAM with an overall adjusted mean difference and 95% CI of 29.1 (-3.44, 61.6) mg/m^2 BSA min^{-1}, $p=0.08$. Adjusting for IHL and VAT in the same model resulted in significantly lower insulin sensitivity in BAM with an overall adjusted mean difference and 95% CI of 42.8 (13.2, 72.4).

Similarly, adjusting whole-body insulin sensitivity expressed as a function of insulin (M/I; (mg/m^2 BSA min^{-1})/(pmol/l)) for VAT, resulted in lower whole-body insulin sensitivity in BAM. The unadjusted overall mean difference and 95% CI between BAM and WEM was not significant at 0.029 (-0.074, 0.131) (mg/kg FFM min^{-1})/(pmol/l), however the overall VAT adjusted mean difference and 95% CI was 0.11 (0.003, 0.18) (mg/kg FFM min^{-1})/(pmol/l), $p<0.01$. Adjusting whole body insulin sensitivity expressed as a function of insulin for IHL alone then IHL and VAT resulted in lower insulin

sensitivity in BAM compared to WEM with an adjusted mean difference and 95% CI of 0.095 (0.017, 0.174) (mg/kg FFM min^{-1})/(pmol/l) p=0.02 and 0.126 (0.054, 0.199) p<0.01 , respectively.

4.5 Discussion

The analyses presented in this chapter assessed the effect of ethnicity on whole-body insulin sensitivity, as measured from a hyperinsulinaemic-euglycaemic clamp. Black west African and white European men showed no evidence of an ethnic difference in whole-body insulin sensitivity in any glucose tolerance group. This disproves the hypothesis of pronounced insulin resistance in black men without T2D compared to their white counterparts but agrees with the hypothesis of similar insulin sensitivity in men with T2D. When adjusting for ethnic differences in visceral adipose tissue, whole-body insulin sensitivity was lower in black men. This may suggest that the lower visceral fat is protecting black men from displaying greater whole-body insulin resistance compared to white men assuming that high volumes of VAT has a causal role in insulin resistance.

The finding of similar whole-body insulin sensitivity in black and white men conflicts with the consensus that black communities are more insulin resistant than their white counterparts (265). Population-based studies assessing a large number of participants, using less sensitive surrogate insulin sensitivity assessments, show pronounced insulin resistance in black participants (256, 282-284). However, participants are not consistently matched for BMI with black participants having a greater BMI, particularly in females, with a BMI difference of between 4 and 5.56kg/m^2 and an average body weight difference of 12kg and 24% more participants defined as obese by BMI (256, 282, 284). A high BMI is a surrogate indicator of greater fat mass which has been associated with insulin resistance (364). Given the association between adiposity and insulin resistance (26, 182), greater BMI in black participants may have confounded the insulin sensitivity findings. In addition to surrogate insulin sensitivity assessments, the intravenous glucose tolerance test (IVGTT) with minimal modelling analysis has been used extensively in black and white communities; it has been described as a robust

estimate of insulin sensitivity. One of the earliest and most compelling reports of ethnic differences in insulin resistance was based on the Insulin Resistance Atherosclerosis Study which assessed 288 African-Americans and 229 non-Hispanic white Americans using the frequently sampled IVGTT with minimal modelling analysis (289, 291). Again, participants were not matched for BMI by ethnicity such that black participants were heavier than white participants with an average BMI difference 1.7kg/m^2. Recent data from clamp studies have also shown lower or a trend towards lower insulin sensitivity in black compared to white participants (319-321). However, yet again, participants were not matched for BMI such that the black participants were more obese than white participants with a mean BMI difference between 2.9 and 4.0kg/m^2. Overall ethnic differences in BMI may have confounded the insulin sensitivity findings in the literature leading to a misinterpretation of insulin resistance in black populations. In order to avoid this potential confounding factor, participants in this study were recruited to be similar in BMI. These data, therefore, contribute to the literature by suggesting that when matched for BMI, ethnic differences in insulin sensitivity between black and white men are absent. Despite the importance which has been placed on BMI matching, studies have criticised BMI for overestimating adiposity in black communities such that for the same BMI, black participants have less fat percent (365). Ethnic-specific BMI cut-offs for obesity have been suggested with a lower cut off point for black participants because the data show that lower BMI thresholds are required in black participants to produce the same diabetes incidence level found in white communities (357). Given that body fat percentage is lower per unit of BMI in black communities; the higher BMIs in the black participants presented in the literature may have resulted in the white and black participants being matched for fat content and therefore the difference in BMI would not confound the insulin sensitivity interpretations. Future studies matching directly for fat content may be more appropriate

when comparing insulin sensitivity in black and white participants to minimise the limitations of matching for BMI which does not accurately reflect body composition or predict fat mass.

Prior studies in the literature have also emphasised that pronounced insulin resistance may be an early defect in black populations thought to explain the disproportionate risk and prevalence. This is supported by lower insulin sensitivity found in black compared to white healthy children and adults (248, 289, 292-302). In comparison, this study showed no ethnic difference between participants with normal glucose tolerance or impaired glucose tolerance which contradicts the hypothesis of early insulin resistance in black men, and also suggests pronounced early insulin resistance may not explain the greater prevalence in black communities.

The discrepancies between the findings from this chapter and the literature may result from differences in the methodologies used to measure insulin sensitivity. This study utilises the hyperinsulinaemic-euglycaemic clamp (156) whereas the evidence for pronounced insulin resistance in black communities has primarily been driven by studies which assess insulin sensitivity using surrogate indices and the IVGTT (265, 285, 286). Insulin sensitivity measures derived from surrogate indices and the IVGTT are based on mathematical models of ambient insulin and glucose concentrations. Pisprasert and colleagues conducted a validation study assessing insulin sensitivity using the clamp and surrogate indices and their findings criticised the use of surrogate indices in black populations (307). They assessed and compared 99 African Americans and 141 European Americans and found no ethnic difference in insulin sensitivity from the hyperinsulinaemic-euglycaemic clamps; however, surrogate indices indicated that black participants had lower insulin sensitivity. The authors discussed that hyperinsulinaemia, a commonly observed characteristic of black communities, may alter the mathematical

model in such a way that it underestimates insulin sensitivity in black populations (307). The circulating insulin concentration is in part determined by insulin secretion and insulin clearance, both of which have not been assessed during the hyperinsulinaemic-euglycaemic clamp. Interestingly, the black participants in this analysis were not more hyperinsulinaemic at baseline or during the low dose insulin infusion and the increment increase in insulin concentration was not ethnically different. This may have been the result of prolonged fasting as the study protocol involved a 2-hour isotope infusion period prior to the clamp. The hyperinsulinemia presented in black compared to white communities is particularly present during obesity (323) rather than in lean participants (322). However, there was a trend towards a higher insulin concentration during the high dose steady-state in NGT which may reflect exaggerated hyperinsulinaemia to a degree. This hyperinsulinaemia is thought to be due to reduced insulin clearance (266, 366, 367). The hyperinsulinaemic-euglycaemic clamp suppresses endogenous glucose production which would usually contribute to the circulating insulin. In comparison, other surrogate methods for assessing insulin sensitivity do not halt this natural feedback loop showing that what we are measuring depends on the tools that we use to measure it.

Overall, the validation study suggests interpretations of insulin sensitivity from indirect assessments should be made with caution and may explain the contrasting findings reported in this analysis. In contrast, it could also be interpreted that the surrogate indices assess insulin sensitivity in a more physiological setting. The insulin and glucose concentrations are based on basal and post load responses, in comparison to the hyperinsulinaemic-euglycaemic clamp which creates a supraphysiological controlled environment. Thus, both the clamp and surrogate measures are valuable tools to assess insulin sensitivity.

Whilst different methods have been proposed to explain the differences in findings from this study in comparison to the literature, other hyperinsulinaemic-euglycaemic clamp studies have shown ethnic differences in insulin sensitivity (245, 251, 311, 312, 319-321). This implies that methodological differences alone, are not the sole explanation for the conflict in findings. Other clamp studies which have analysed men in isolation, who were not consistently matched for BMI, have reported no ethnic difference in whole-body insulin sensitivity (307, 313). Another study (*Stull et al.*) has provided evidence for greater whole-body insulin sensitivity in non-diabetic black compared to white men matched for BMI (312). Participant adiposity assessments could not explain the conflicting finding from *Stull et al.* compared to the finding of no ethnic difference in this study. The sample size was uneven and smaller in the report by *Stull et al.* (African American n = 4, Caucasian n = 35), thus statistical power may explain the conflicting results. The finding of no ethnic difference in whole-body insulin sensitivity from this study also contrasts with the majority of studies in obese women without T2D, where lower insulin sensitivity in black women is reported (245, 251, 311). This may imply that the lower whole-body insulin sensitivity in black compared to white participants is restricted to women and not men. It may also be evidence that the effect of ethnicity depends on obesity, such that ethnic differences are present during obesity. Thus, the ethnic difference in whole-body insulin sensitivity may be sex-specific, obesity-specific or a combination. In support of a sex-specific response, studies have shown sex differences in insulin sensitivity in black populations; black women are more insulin resistant and hyperinsulinaemic than black men (353, 368). Studies have also reported sex differences in body composition such that black women have less visceral adiposity and more subcutaneous fat compared to black men (369). These sex differences in the

insulin resistance, hyperinsulinemia and body composition in black populations suggest that when compared to white populations, sex differences may be plausible.

Despite a potential sex effect which may explain why similar insulin sensitivity was found in this study of men and greater resistance has been shown in black women; other clamp studies have found no ethnic differences in whole-body insulin sensitivity in women (246, 312). Therefore, the sex explanation is not consistent in all publications. The similar whole-body insulin sensitivity in black and white men shown in this chapter aligns with some of the clamp studies in the literature which assess a range of glucose tolerances, BMIs, both men and women (307, 309, 312-319) and men alone (307, 313). In some of the previous studies, BMI was not consistently matched such that black participants were heavier (307, 317) which may have confounded their results. Therefore, this study contributes to the literature by suggesting that the lack of an ethnic difference between black and white men is true in each glucose tolerance group when matching for BMI.

As discussed in chapter 1, there are multiple methods for expressing whole-body insulin sensitivity from a hyperinsulinaemic-euglycaemic clamp. Total glucose disposal during insulin stimulation is used to measure whole-body insulin sensitivity, and this must be normalised by expressing the data per unit of metabolic body size for comparison between groups (33, 84, 104). Normalising whole-body insulin sensitivity using body weight has been shown to underestimate insulin sensitivity in obesity (33). In this chapter, the main effect of ethnicity was not statistically different regardless of whether whole-body insulin sensitivity was expressed by body weight, body surface area (BSA) or fat-free mass (FFM). Thus, further chapters in this thesis will express insulin data per m^2 BSA.

It has also been suggested that total glucose disposal could be expressed as a factor of insulin to represent whole-body insulin sensitivity (156). In this study, although the same insulin infusion was applied to all participants, BAM with NGT exhibited a trend towards greater insulin concentrations during the high dose insulin infusion. This may be due to reduced insulin clearance (266, 366) and would suggest that insulin adjustments are appropriate to eliminate this confounding factor. Some authors argue that measures must be adjusted and expressed per unit of insulin (89) although, the adjustment for insulin relies on the assumption that the relationship with glucose is linear (89), other investigators criticise insulin adjustments for increasing variability (104). To assess whether the relationship between insulin and glucose was linear, a correlation analysis was conducted. In both ethnic groups, data showed a significant linear relationship between basal glucose and insulin in men without diabetes but no relationship in T2D. This finding agrees with the literature which shows a non-linear inverted "U" relationship between fasting insulin and glucose during the progression to T2D; defined as starlings curve of the pancreas (76). Initially, as insulin resistance rises, the insulin response from the beta cell increases to offset the disruption in glucose metabolism which increases insulin concentrations. Once the beta-cell fails to augment this accelerated insulin secretory capacity, glucose tolerance declines. Further increases in glycemia occur in the presence of declining insulin concentrations which explains why individuals with T2D can have insulin concentrations similar to those with NGT (72, 76, 370, 371). The lack of linearity in T2D suggests that participants may be at different points along the inverted "U" insulin curve, thus insulin adjustment may not be appropriate because glucose and insulin are not associated. This is further supported by the highly variable insulin concentrations found in men with T2D (104, 372). Overall, whilst there is debate as to whether insulin sensitivity should be adjusted for insulin, data from these analyses

showed no ethnic difference in whole-body insulin sensitivity with or without insulin adjustments. Both approaches will be assessed in subsequent chapters.

The accumulation of central adiposity has consistently been linked with insulin resistance (182, 201, 363). Despite the greater risk of developing T2D in black populations, they have repeatedly been reported to present with lower visceral adipose tissue in the presence of greater insulin resistance, which does not align with the current views of T2D pathophysiology (247, 289). Data in this chapter showed that black men have similar whole-body insulin sensitivity in the presence of lower VAT (chapter 3). When adjusting whole-body insulin sensitivity for VAT, black men had lower insulin sensitivity compared to white men. This may suggest that the comparable whole-body insulin sensitivity can be explained partially by lower VAT in black men. The lower VAT was found regardless of glucose tolerance; thus lower VAT accumulation may protect black men from being more insulin resistant. In support of the concept that lower VAT may be protecting black men from being more insulin resistant, a study which assessed obese black and white women with similarly high levels of VAT, contradictory to the ethnicity literature (because black participants usually present with low VAT), showed lower insulin sensitivity in black compared to white women (251). Another study which assessed black and white women with similar waist to hip ratios, an indication of central adiposity, also showed lower insulin sensitivity in black women (311). These conclusions and data in this chapter may imply that having lower VAT may protect black men from presenting with pronounced insulin resistance compared to white men.

Individuals with insulin resistance and T2D have often been reported to present with fatty liver (222). Lalia and colleagues assessed 116 non-diabetic men and women across a range of BMIs using pancreatic hyperinsulinaemic-euglycemic clamps to assess whole body insulin sensitivity, proton magnetic resonance spectroscopy to assess

intrahepatic lipids (IHL) and magnetic resonance imaging to assess VAT. They found using correlations and multiple linear regression that both IHL and VAT associate with and are significant predictors of whole-body insulin sensitivity (373). The data in this chapter show that adjusting for the ethnic differences in IHL results in trend towards insulin sensitivity being lower in black compared to white men. This finding may suggest that presenting with lower hepatic fat may also protect black men from displaying pronounced insulin resistance.

Assessing whole-body insulin sensitivity (total glucose disposal) during a hyperinsulinaemic-euglycaemic clamp does have its limitations. It assumes that endogenous glucose production is completely suppressed during the insulin infusion (156). However, in cases of insulin resistance and type 2 diabetes, an insulin infusion of 40mU/m^2BSA/min has been shown to cause incomplete suppression of endogenous glucose production (87, 163). Thus, endogenous glucose production, as well as the exogenous glucose infusion, contribute to the glucose entry into the circulation and the M value underestimates insulin sensitivity in these groups. This measurement limitation can be alleviated by using stables isotopes to directly trace glucose entry and removal from the circulation (159). Ethnic differences may occur in the suppression of endogenous glucose production and have been published (323), which may therefore disproportionately influence an ethnic comparison of the M value which is not able to capture the endogenous glucose production without stable isotopes.

To conclude, this chapter aimed to assess ethnic differences in whole-body insulin sensitivity in black west African and white European men across a range of glucose tolerances. By using the hyperinsulinaemic-euglycaemic clamp to measure total glucose disposal, there were no ethnic differences in insulin sensitivity in any glucose tolerance group using multiple expressions of whole-body insulin sensitivity. This finding occurred

in the presence of lower visceral fat, adjusting for which, reduces insulin sensitivity in black men, suggesting that lower central fat accumulation contributes to the comparable insulin sensitivity.

Chapter 5: A comparison of peripheral insulin sensitivity between black and white men

Chapter highlights:

- There were no ethnic differences in peripheral insulin sensitivity. This finding was consistent in all glucose tolerance groups.
- Similar peripheral insulin sensitivity occured in the presence of lower VAT in black men.
- There was some evidence to suggest that lower VAT in black men may protect them from having greater peripheral insulin resistance.

Data presented in this chapter have been published:

Bello O, Mohandas C, Shojaee-Moradie F, Jackson N, Hakim O, Alberti KGMM, Peacock JL, Umpleby AM, Amiel SA, Goff LM. Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat. Diabetologia. 2019 May;62(5):835-844.

Bello O, Ladwa M, Hakim O, Marathe C, Shojaee-Moradie F, Charles-Edwards G, Peacock JL, Umpleby MA, Amiel S, Goff LM. Differences in the link between insulin sensitivity and ectopic fat in men of Black African and White European ethnicity. Eur J Endocrinol. 2019 Nov;182 (1):91-101

5.1 Introduction

Insulin has a direct action on multiple sites which include skeletal muscle and adipocytes, collectively termed peripheral tissue (95). Insulin has been well described as a stimulator of peripheral glucose disposal where it increases glucose uptake and influences both oxidative and non-oxidative glucose metabolism. These actions lead to a reduction in circulating glucose (95). During the insulin-stimulated state, the majority of glucose is disposed of in peripheral tissues, and almost all peripheral glucose disposal occurs in the skeletal muscle (76, 90, 95, 97, 103-105) with relatively little in the adipose tissue (95-99). Thus, peripheral insulin sensitivity is primarily an assessment of skeletal muscle insulin sensitivity (95). Studies have shown over 45% lower insulin-mediated skeletal muscle glucose disposal in participants with obesity (98, 374) or type 2 diabetes (T2D) (75, 86, 90, 97) compared to healthy controls. This large difference supports the conclusion drawn by multiple investigators that identify skeletal muscle insulin resistance as a primary contributor to T2D (95). The hyperinsulinaemic-euglycaemic clamp enables quantification of total glucose disposal, the M value, which is derived from an exogenous glucose infusion. As discussed in chapter 1 (section 1.6) and chapter 4 (section 4.5), this procedure assumes that hyperinsulinaemia causes complete suppression of any endogenous glucose production, which is not the case in individuals with more pronounced insulin resistance (87, 163). In these cases, the M value can underestimate peripheral insulin sensitivity. The use of stable isotope tracers allows for a more direct assessment of glucose disposal by measuring the glucose disappearance, which removes the limitation of the M value. During insulin stimulation, glucose disposal occurs primarily in the peripheral tissues, thus when placed in the context of hyperinsulinaemia, the glucose disappearance can be used as an assessment of peripheral insulin sensitivity.

Insulin sensitivity has been consistently linked to adiposity (particularly central adiposity) such that high levels of adiposity are associated with low insulin sensitivity (182). Increases in visceral adipose tissue, in particular, have been associated with an increased risk of insulin resistance and type 2 diabetes (186). Where peripheral insulin sensitivity has been assessed using isotopic tracers, a significant association with visceral adipose tissue has been identified (203-205). Theories which may explain this association have been discussed in chapter 1 (section 1.7.1).

Studies in black and white adults, which assess peripheral insulin sensitivity using glucose isotopic tracers, have been restricted to women. The first of which was conducted by DeLany *et al.* in non-diabetic lean women who were matched for age and BMI which was on average 23 years and 22.7 kg/m^2, respectively. The participants also presented with similar levels of free-living physical activity assessed as time in vigorous activity, time in moderate activity and steps per day. The data presented by DeLany *et al.* showed peripheral insulin sensitivity to be 26% lower in African-American women compared to white women (322). Since then, a single study in obese women has been conducted by Goedecke and colleagues. They assessed south African black and white women matched for age and BMI which was on average 36 years and 36.6 kg/m^2, respectively, and found no ethnic differences in peripheral insulin sensitivity (323). This study included participants with impaired glucose tolerance and normal glucose tolerance; however, their data were not presented by glucose tolerance. The interpretation of these results may imply that lower insulin sensitivity in black compared to white women occurs only in a lean state. Whether these findings can be extrapolated to men and whether it occurs in all glucose tolerance groups is unknown.

5.2 Aim

This chapter aims to determine whether there are ethnic differences in peripheral insulin sensitivity to glucose metabolism between black west African (BAM) and white European men (WEM) with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or early type 2 diabetes (T2D).

It could be hypothesised that BAM will have lower peripheral insulin sensitivity compared to WEM.

5.3 Methods

A full description of the study methods including the participant inclusion criteria, metabolic assessments and insulin sensitivity calculations is provided in chapter 3. In summary, black west African (BAM) and white European (WEM) participants were categorised as normal glucose tolerant (NGT) or impaired glucose tolerant (IGT) based on their oral glucose tolerance test, or as having type 2 diabetes (T2D) if diagnosed by their primary care practitioner within 5 years.

Eligible participants attended a two-step hyperinsulinaemic-euglycaemic clamp with a stable [6,6 $^{2}H_{2}$]-glucose isotope infusion as a direct assessment of peripheral glucose disposal (glucose rate of disappearance (Rd)) for peripheral insulin sensitivity measures. The primed continuous infusion of [6,6 $^{2}H_{2}$]-glucose isotope was initiated at time -120 minutes and ran until the end of the procedure at time 240 minutes. Basal assessments of the peripheral glucose disposal rate were made using samples drawn from time -30 to 0 minutes. Subsequently, at time point 0 minutes an insulin infusion was initiated at 10mU/m^{2}BSA/min and 40mU/m^{2}BSA/min for 2 hours each, this defined the two-steps of the hyperinsulinaemic-euglycaemic clamp. Peripheral glucose disposal during the high dose insulin infusion was assessed from samples drawn during the last 30 minutes of the high dose insulin infusion. At this point, a steady-state equilibrium is achieved whereby the glucose infused to maintain euglycemia (5mmol/l) equilibrates to the total glucose disposal from the circulation.

Participants also attended a magnetic resonance imagining scan for assessment of visceral adipose tissue (VAT) area at the L4/5 anatomical position. Intrahepatic lipid (IHL) was also assessed as the average hepatic fat fraction from 8 circular regions of interests from the superior and inferior surface of the liver.

5.3.1 Calculations

Peripheral glucose disposal (rate of disappearance, Rd) at basal and during the high dose insulin infusions were expressed per m^2 body surface area (m^2 BSA). Peripheral insulin sensitivity was assessed as the percentage increase in peripheral glucose disposal rate from basal to the steady-state of the high dose insulin infusion (224, 239, 343). The relatively small contribution of adipose tissue to glucose uptake suggests that the rise in peripheral glucose disposal is a measure of skeletal muscle insulin sensitivity (97). The peripheral insulin sensitivity index (PISI) was also calculated during the steady-state of the high dose insulin infusion. This was computed as the peripheral glucose disposal divided by the peripheral insulin concentration (153).

5.3.2 Statistical analyses

Data were assessed for normality using the Shapiro-Wilks test and histograms. Parametric data are presented as mean (SD), data which required transformation (log 10) are presented as geometric mean (95% CI) and data which remained non-parametric are presented as median (interquartile range). A two-way analysis of variance (ANOVA) was conducted to assess the main effect of ethnicity, glucose tolerance and the interaction. This was conducted on parametric data and non-parametric data however, the ethnicity findings for the latter were confirmed with Mann-Whitney U tests. To interpret the ANOVA, the interaction statistic was first checked before assessing the main effect of ethnicity and glucose tolerance. Where the interaction statistic was non-significant, the main effect of ethnicity and glucose tolerance were independent of one another, thus interpreted in isolation. There were more than 2 glucose tolerance groups (NGT, IGT, T2D), therefore when a significant main effect of glucose tolerance was found, Tukey's post hoc tests were employed to determine where the differences lay. To control for ethnic

differences in VAT shown in chapter 3 (section 3.2.2), linear regression was used to assess the effect of ethnicity on peripheral insulin sensitivity whilst adjusting for glucose tolerance, VAT and IHL. These data are reported as the unadjusted and adjusted mean difference and 95% CI (WEM minus BAM). Statistical significance was defined as $p < 0.05$. Data analyses were performed using SPSS software, version 25 (IBM Analytics, Armonk, NY, USA).

5.4 Results

A full description of the characteristics of the participants analysed in this chapter has been presented in chapter 3. In summary, participants were between the ages of 18-65 years with a BMI between 20 – 40 m^2/kg. Within each glucose tolerance group, the age of BAM was lower compared to WEM, particularly in those with IGT ($p=0.02$). There were no ethnic differences in BMI in any glucose tolerance group ($p=0.93$), and on average, the majority of the men were overweight or obese. Visceral and hepatic fat were also significantly lower in BAM compared to WEM ($p<0.01$ and $p<0.01$, respectively). A full description of the plasma glucose and insulin profiles during the clamp are shown in chapter 4 (section 4.4.1). A trend towards an ethnic difference was found in participants with NGT, whereby BAM had higher insulin concentrations during the steady-state of high dose insulin infusion.

5.4.1 Peripheral glucose disposal

Peripheral glucose disposal (Glucose Rd) was measured at basal and during the high dose insulin infusion (table 5). The results of the two-way ANOVA showed no statistically significant main effect of ethnicity on basal or insulin stimulated peripheral glucose disposal (table 5). As expected, there was a statistically significant main effect of glucose tolerance for all measures of peripheral glucose disposal. During the basal state mean peripheral glucose disposal expressed was higher in NGT compared to T2D ($p<0.01$) but not different from IGT ($p=0.48$) and there was no difference between IGT and T2D ($p=0.31$). During the high dose insulin infusion, peripheral glucose disposal was statistically higher in men with NGT compared to those with IGT and T2D ($p<0.01$) however, there was no statistical difference between IGT and T2D men ($p>0.97$).

5.4.2 Peripheral insulin sensitivity

Peripheral insulin sensitivity was assessed using the peripheral insulin sensitivity index (PISI) during the high dose insulin infusion, which expresses peripheral glucose disposal as a function of insulin (table 5, figure 13A). The two-way ANOVA shows no statistically significant effect of ethnicity on PISI however, as expected, there was an effect of glucose tolerance. PISI was higher in NGT compared to IGT or T2D ($p<0.01$) but there was no difference between participants with IGT or T2D ($p=0.96$).

Peripheral insulin sensitivity was also calculated as the percentage increase in peripheral glucose disposal from basal to the high dose insulin infusion. This measure accounts for differences in basal metabolism and the change in insulin (table 5, figure 13B). The outcome of the two-way ANOVA showed no ethnic difference in peripheral insulin sensitivity ($p=0.22$). As expected, there was a statistically significant effect of glucose tolerance. Participants with NGT had higher peripheral insulin sensitivity than those with IGT or T2D ($p<0.01$) and there was no difference between participants with IGT or T2D ($p=0.78$).

Table 5: *Peripheral glucose disposal and peripheral insulin sensitivity before and during the hyperinsulinaemic-euglycaemic clamp*

	NGT		IGT		T2D				
	BAM 21	**WEM 23**	**BAM 10**	**WEM 9**	**BAM 18**	**WEM 15**	P_1 **Ethnicity**	P_2 **Glucose tolerance**	P_3 **Interaction**
Basal steady state									
Peripheral glucose disposal; μmol/m^{2}BSA min^{-1}	451.7 (47.2)	450.7 (45.2)	426.5 (61.5)	441.2 (37.7)	405.6 (64.0)[d]	413.4 (77.6)[d]	0.57	0.01	0.86
High dose insulin infusion steady state									
Peripheral glucose disposal; μmol/m^{2}BSA min^{-1}	1666.4 (1482.0–1958.0)[ab]	1759.1 (1225.7–2195.1)[b]	1175.6 (285.9)	1069.2 (328.1)	1134.2 (418.5)	1037.7 (346.1)	0.47	<0.01	0.94
Peripheral insulin sensitivity index; (μmol/m^2 BSA min^{-1})/(pmol/l)	3.18 (1.13)[a]	3.51 (1.76)	1.92 (0.56)	1.80 (0.71)	2.03 (1.06)	1.78 (0.58)	0.96	<0.01	0.54
Increase in peripheral glucose disposal (%)	304.8 (111.1)[a]	286.2 (138.4)	183.2 (86.0)	142.1 (70.1)	203.5 (126.2)[d]	166.2 (102.5)[d]	0.22	<0.01	0.92

Data are expressed as mean (SD) for parametric data and median (IQR) for non-parametric data
P_1 : Main effect of ethnicity , P_2 : Main effect of glucose tolerance, P_3 : Ethnicity and glucose tolerance interaction
[a]Sample size: BAM=20, [b]Median (IQR), [c]Sample size: BAM=20, [d]Sample size: BAM=15, WEM=12.
The percentage increase in peripheral glucose disposal was assessed from basal to high dose insulin infusion steady state.
Abbreviations: NGT - normal glucose tolerance, IGT - impaired glucose tolerance, T2D - type 2 diabetes, BAM - black west African men, WEM - white European men

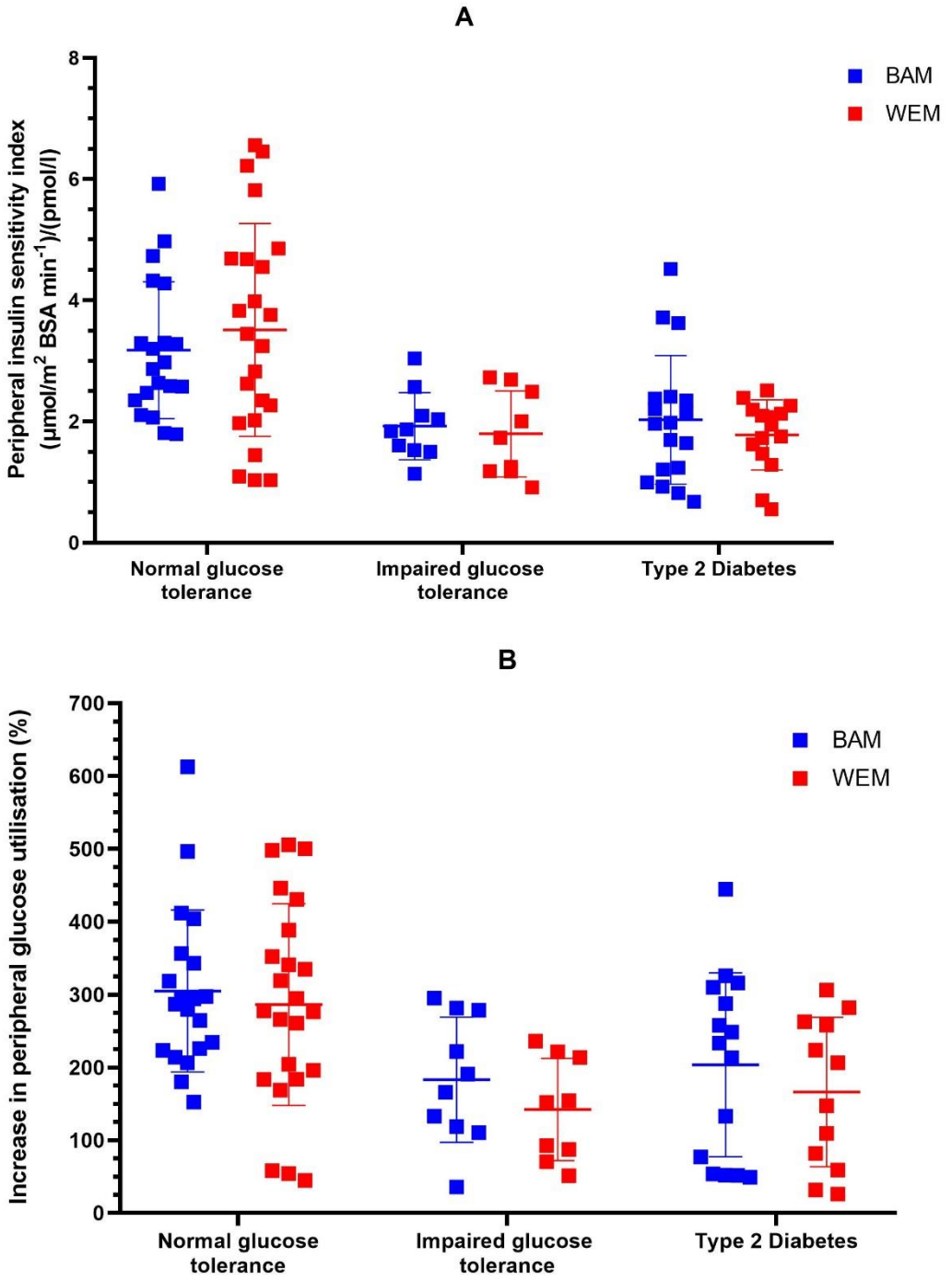


Figure 13: *Peripheral insulin sensitivity in black west African (BAM) and white European men (WEM) by glucose tolerance.*

Peripheral insulin sensitivity calculated as A) peripheral glucose disposal as a function of insulin during the high dose infusion steady-state and B) the percentage increase in peripheral glucose disposal from basal to high dose insulin infusion steady-state.
Data expressed as mean (SD).
Abbreviations: BSA – body surface area

5.4.3 Peripheral insulin sensitivity adjustment for VAT and IHL

Ethnic differences in VAT and IHL were observed whereby VAT and IHL were lower in BAM compared to WEM as shown in chapter 3 (section 3.4.2).

The overall unadjusted mean difference and 95%CI of the percentage increased in peripheral glucose disposal between BAM and WEM was -20.0 (-75.3, 35.3) %, p=0.47, peripheral insulin sensitivity was higher in BAM however this was not statistically significant. Adjusting the percentage increase in peripheral glucose disposal for VAT resulted in lower peripheral insulin sensitivity in BAM in the overall cohort. Again, this did not reach statistical significance with an unadjusted mean difference and 95% CI of 22.9 (-20.0, 65.8)%, p=0.29. Adjusting the percentage increase in peripheral glucose disposal for IHL resulted in an adjusted mean difference and 95% CI of 12.6 (-33.5, 58.6) %, p=0.59. When adjusting for VAT and IHL there was no significant difference with an adjusted mean difference and 95% CI of 30.6 (-11.7, 73.0), p=0.15.

PISI was also used as an assessment of peripheral insulin sensitivity. The overall unadjusted mean difference and 95%CI in PISI between BAM and WEM was 0.15 (-0.43, 0.72) ($\mu mol/m^2$ BSA min^{-1})/(pmol/l), p=0.62. Adjusting PISI for VAT resulted in significantly lower peripheral insulin sensitivity in BAM compared to WEM with an overall adjusted mean difference and 95% CI of 0.58 (0.16, 1.00) ($\mu mol/m^2$ BSA min^{-1})/(pmol/l), p<0.01. Adjusting PISI of IHL also resulted in significantly lower peripheral insulin sensitivity in BAM compared to WEM with an overall adjusted mean difference and 95% CI of 0.51 (0.07, 0.95) ($\mu mol/m^2$ BSA min^{-1})/(pmol/l), p=0.03. Adjusting PISI for VAT and IHL, resulted in significantly lower peripheral insulin sensitivity in BAM compared to WEM with an overall adjusted mean difference and 95% CI of 0.68 (0.27,1.10) ($\mu mol/m^2$ BSA min^{-1})/(pmol/l), p<0.01.

5.5 Discussion

In this study, peripheral glucose disposal was assessed in black west African and white European men of various glucose tolerances. This was assessed using a two-step hyperinsulinaemic-euglycaemic clamp with a glucose isotope tracer for a more direct quantification for peripheral glucose disposal and peripheral insulin sensitivity. There was no evidence for an ethnic difference in peripheral glucose disposal or peripheral insulin sensitivity in men of normal glucose tolerance, impaired glucose tolerance or type 2 diabetes. This disagrees with the hypothesis of lower peripheral insulin sensitivity in black compared to white men.

There are multiple reports for greater insulin resistance in black compared to white populations which are generally described to reflect whole-body or peripheral insulin sensitivity (265, 285, 286). A defect in peripheral tissues response to insulin is thought to be an early sign of the progression to T2D (95). Whilst assessments of whole-body insulin sensitivity are primarily a reflection of peripheral tissue glucose disposal, using isotopic tracers are a more accurate method to assess peripheral glucose disposal (159). This is the first analysis to directly compare peripheral insulin sensitivity in black and white men using isotopic tracers and a hyperinsulinaemic-euglycaemic clamp. It is also the first study to assess peripheral insulin sensitivity in black and white participants over a range of glucose tolerances. Prior literature which use isotope methods to measure peripheral insulin sensitivity in black and white populations are limited to women (322, 323). In agreement with the findings in this analysis, a single study in obese non-diabetic women matched for BMI and age, on average 36.6 kg/m^2 and 36 years respectively, by *Goedecke et al.* also found no ethnic difference in peripheral insulin sensitivity (323). This occurred using the same protocol (insulin infusion, insulin duration and glucose isotope) as this study; however, their data analysis combined participants with normal glucose tolerance

and impaired glucose tolerance. *Goedecke et al.* were therefore unable to assess a potential ethnic difference in both glucose tolerances. This study showed no ethnic difference in peripheral insulin sensitivity men in each glucose tolerance group and contributes to the literature by suggesting that black and white men have similar peripheral insulin sensitivity across the spectrum of glucose intolerance and potentially during the disease progression. These data also suggest that the early peripheral insulin resistance is not present in black men and the peripheral insulin response is not likely to contribute to the ethnic-specific acceleration of T2D risk.

However, the finding of similar peripheral insulin sensitivity does not agree with *Delany et al.*, who studied lean normal glucose tolerant women who were matched for BMI and age which was on average 22.7kg/m^2 and 23.6 years, respectively. Using the same protocol (insulin infusion, insulin duration and glucose isotope), they found lower peripheral insulin sensitivity in black compared to white women (322). The conflicting findings are not likely to reflect sex differences as data in this chapter agree with *Goedecke et al.* (323) who studied obese women. The conflict with *Delany et al.* may, therefore, be explained by the BMI status of participants. The participants studied by *Delany et al.* were significantly leaner, the average BMI difference was 13kg/m^2, compared to this study or *Goedecke et al*. It's possible that increased insulin resistance in black women may be present in the younger, leaner healthy state which supports the notion of early insulin resistance shown in individuals without T2D (289, 297, 299-302). However, the presence of obesity may outweigh or attenuate any ethnic differences, which has been discussed in another study (375). The relationship between insulin sensitivity and measures of adiposity may be ethnically distinct as supported by the continuous finding of insulin resistance in the presence of lower ectopic fat. An ethnic distinction in the relationship between adiposity and insulin sensitivity has been identified

by a study in black and white South African women which showed a weaker relationship in black compared to white women (255). White participants may, therefore, be more susceptible to obesity-related insulin resistance such that, during obesity insulin resistance becomes comparable to that seen in black participants, hence why similar peripheral sensitivity may have been found in obese black and white women in Goedecke *et al.* (323).

The method used by *Delany et al*. to assess peripheral insulin sensitivity may also explain the contrasting findings with this study. They did not comment on whether residual endogenous glucose production was accounted for when measuring peripheral insulin sensitivity and their methods describe the M value, which is generally used to assess whole-body insulin sensitivity (peripheral glucose uptake and suppression of endogenous glucose production). Whilst this may be a potential factor to consider, their participants were lean and insulin-sensitive; therefore, it could be assumed that endogenous glucose production was completely suppressed, and only peripheral glucose uptake was captured.

One of the strengths of this analysis is the assessment of peripheral insulin sensitivity using the percentage change in peripheral glucose disposal (239) and the peripheral insulin sensitivity index (PISI) (153). There is no consensus on the most appropriate way to express peripheral insulin sensitivity from a hyperinsulinaemic-euglycaemic clamp with isotopic tracers; both methods are employed throughout the literature. When peripheral insulin sensitivity is compared between groups, in cases where insulin concentrations between groups are similar, peripheral or total glucose disposal in response to insulin is compared without an insulin adjustment. However, insulin has a dose-dependent effect on glucose, thus adjustments for insulin are often used (156). In this study, although participants were infused with equal doses of insulin, there

was a trend towards a higher insulin concentration during the steady-state of the high dose infusion in black men with normal glucose tolerance compared to white men shown in chapter 4 (section 4.4.1). These data suggested that an insulin adjustment may have been warranted and was made directly using the PISI and indirectly using the percentage increase in peripheral glucose disposal in response to insulin. Both assessments showed no ethnic difference thus did not change the ethnicity finding. Finally, by assessing whole-body (chapter 4) and peripheral insulin sensitivity in the same cohort of participants, the findings suggest that ethnic differences are absent whether the glucose infusion or more accurate isotopes are used to quantify peripheral glucose disposal.

Central adiposity, particularly visceral adipose tissue (VAT), has been associated with insulin resistance and T2D. Studies have reported a significant association between peripheral insulin sensitivity and VAT, where it has been implied that increasing VAT increases peripheral insulin resistance (203-205). Details for the mechanisms which may link VAT to peripheral insulin resistance have been discussed in chapter 1 (section 1.7.1). Black populations have consistently been reported to present with reduced VAT (245-252) which was also found when comparing the men in this cohort shown in chapter 3 (section 3.4.2). This study shows similar peripheral insulin sensitivity in black and white men occurs in the presence of lower VAT in black men. When adjusting PISI for the ethnic differences in VAT, black men presented with significantly lower peripheral insulin sensitivity. This mirrors the finding in chapter 4 (section 4.4.3) whereby adjusting whole-body insulin sensitivity for VAT resulted in lower whole-body insulin sensitivity in black men. The adjustment suggests that lower VAT may protect black men from having greater peripheral insulin resistance. In comparison, using the percentage increase in peripheral glucose disposal as a measure of peripheral insulin sensitivity did not result in an ethnic difference after adjusting for VAT. This finding would suggest that VAT is

not a large contributor to peripheral insulin resistance in black populations, and the association may be less significant. It may also imply that the theories which implicate VAT as having a causal influence of peripheral insulin resistance, are less relevant in black populations who have less VAT, thus would be expected to have lower peripheral insulin resistance. The conflict in the ethnicity findings when adjusting PISI and the percentage increase in glucose disposal for VAT may be due to differences in sample size, thus a power issue. More participants had a measurement of PISI in comparison to the percentage increase in peripheral glucose disposal. Larger studies are required to confirm whether ethnic differences are present in peripheral insulin sensitivity after adjusting for lower VAT in black populations.

Intrahepatic lipids (IHL) have also been suggested to associate with insulin resistance to glucose homeostasis. Fabbrinia and colleagues studied 42 obese men and women without T2D or a history of hepatic abnormalities using magnetic resonance imaging to assess VAT, proton magnetic resonance spectroscopy to assess hepatic fat and Hyperinsulinaemic-euglycaemic clamps to assess insulin sensitivity (224). Using linear regression analyses, they found that hepatic fat was the best predictor of hepatic, skeletal muscle and adipose tissue insulin sensitivity. The data in this chapter show some evidence that adjusting for IHL, which was significantly lower in black men, resulted in lower peripheral insulin sensitivity in black compared to white men. Similar to the adjustment fpr VAT, this finding occurred when correcting PISI and not the percentage change in glucose disposal for IHL. Larger studies are required to confirm whether ethnic differences are present in peripheral insulin sensitivity after adjusting for the lower IHL found in black communities.

To summarise, this chapter aimed to assess whether ethnic differences in peripheral insulin sensitivity are present in black west African and white European men

in multiple glucose tolerance groups. By using multiple direct assessments of peripheral glucose disposal in response to insulin stimulation, these data show no evidence for an ethnic difference in peripheral insulin sensitivity which disproves the original hypothesis. This occurred in each glucose tolerance groups and suggests that peripheral insulin sensitivity alone is, unlikely to explain the high risk of T2D in black compared to white men. Participants were matched for BMI; however, black men presented with lower VAT compared to white men. After adjusting peripheral insulin sensitivity for ethnic differences in VAT, peripheral insulin sensitivity appeared to be lower in black men compared to white men; however, these data were inconsistent. Whether visceral adiposity is equally detrimental to peripheral insulin sensitivity in black and white men cannot be concluded by these data.

Chapter 6: A comparison of hepatic insulin sensitivity between black and white men

Chapter highlights:

- There were no ethnic differences in hepatic insulin sensitivity. This finding was consistent in all glucose tolerance groups.
- Similar hepatic insulin sensitivity occured in the presence of lower visceral and hepatic fat in black men.
- Lower visceral and hepatic fat in black men may protect them from having lower hepatic insulin sensitivity when compared to white men.

Data presented in this chapter have been published:

Bello O, Mohandas C, Shojaee-Moradie F, Jackson N, Hakim O, Alberti KGMM, Peacock JL, Umpleby AM, Amiel SA, Goff LM. Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat. Diabetologia. 2019 May;62(5):835-844.

Bello O, Ladwa M, Hakim O, Marathe C, Shojaee-Moradie F, Charles-Edwards G, Peacock JL, Umpleby MA, Amiel S, Goff LM. Differences in the link between insulin sensitivity and ectopic fat in men of Black African and White European ethnicity. Eur J Endocrinol. 2019 Nov;182 (1):91-101

6.1 Introduction

As discussed in chapter 1 (section 1.5.2), insulin has been described to affect hepatic function through multiple mechanisms, which lead to a reduction in hepatic glucose production (128). During the fasted state, insulin action at the liver is low; the resulting hepatic glucose production provides a fuel source for all organs, particularly neural tissues. In comparison, during the fed state, when insulin concentrations increase, hepatic glucose production is suppressed to prevent excess hyperglycaemia. A reduction in hepatic insulin sensitivity, termed hepatic insulin resistance, has been well documented to be greater in type 2 diabetes and has been implicated in the disease progression (128, 132).

In order to assess hepatic insulin sensitivity, glucose isotopes have been employed, which trace glucose flux (159). The glucose isotopic tracers allow for a direct assessment of glucose appearance, a reflection of total endogenous glucose production. Hepatic glucose production makes up 95% of total endogenous glucose production (130); thus, quantifying endogenous glucose production is used as an assessment of hepatic glucose production. When placed in the context of insulin, this is used to derive hepatic insulin sensitivity. The majority of studies which directly assess hepatic insulin sensitivity do so; by measuring the percentage suppression of endogenous glucose production in response an insulin infusion, by calculating the basal hepatic insulin sensitivity index (HISI) or by calculating the basal hepatic insulin resistance index (HIRI) (204, 344).

Hepatic insulin sensitivity has been tightly associated with visceral adipose tissue (VAT) and intrahepatic fat (IHL), it may potentially influence or be influenced by VAT and IHL (203-205, 226, 227, 373). The potential mechanisms which link VAT and IHL to hepatic insulin resistance have been described in chapter 1 (section 1.7.1 and 1.7.2) where adipokines, cytokines, fatty acids and hepatic diacylglycerol have been implicated.

Studies which assess hepatic insulin sensitivity using isotopic tracers in black and white adults have primarily been conducted in women, in each case the black and white participants were matched for BMI and age. There have been reports of no ethnic difference in hepatic insulin sensitivity either during the basal or insulin stimulated state in obese and lean participants with an average BMI of 32.5kg/m^2 and 22.7 kg/m^2, respectively (246, 322). There have also been reports that obese black women have greater hepatic insulin sensitivity during the stimulated state compared to obese white women with a cohort average BMI of 36.6 kg/m^2 (323). A study which assessed women across a range of BMIs found lower hepatic insulin sensitivity during the basal state in black compared to white women (296). Only a single study has included both male and female participants which covered a range of BMIs; the authors reported no ethnic difference in basal or insulin suppressed endogenous glucose production (315). The inconsistent findings may be due to basal versus insulin stimulated assessment of hepatic insulin sensitivity, the degree of participant obesity, the sex of the participants or the glucose tolerance status of participants.

6.2 Aim

This chapter aims to determine whether there are ethnic differences in hepatic insulin sensitivity between black west African (BAM) and white European men (WEM) of normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or early type 2 diabetes (T2D).

It could be hypothesised, based on lower visceral and ectopic fat in black communities, that BAM will display greater hepatic insulin sensitivity compared to WEM.

6.3 Methods

A full description of the study methods is provided in chapter 2. This includes the participant inclusion criteria, metabolic assessments and the calculations used to derive insulin sensitivity. To summarise, black west African (BAM) and white European men (WEM) were defined as normal glucose tolerant (NGT) or impaired glucose tolerant (IGT) based on their oral glucose tolerance test. Participants with type 2 diabetes (T2D) had a diagnosis of T2D within 5 years documented in their medical records.

Eligible participants attended a two-step hyperinsulinaemic-euglycaemic clamp with a stable [6,6 $^{2}H_{2}$]-glucose isotope infusion to assess endogenous glucose production (glucose rate of appearance (Ra)) which was used to calculate hepatic insulin sensitivity. A primed continuous infusion of [6,6 $^{2}H_{2}$]-glucose isotope was initiated at time -120 minutes and ran until the end of the procedure at time 240 minutes. A basal assessment of endogenous glucose production was made using samples drawn from time -30 to 0 minutes by which time the [6,6 $^{2}H_{2}$]-glucose isotope infusion had achieved an equilibrium enrichment with plasma glucose. Subsequently at time point 0 minutes, an insulin infusion was initiated at 10mU/m^{2}BSA/min and 40mU/m^{2}BSA/min for 2 hours each, which defined the two-steps of the hyperinsulinaemic-euglycaemic clamp. Endogenous glucose production during the low and high dose insulin infusion was assessed from samples drawn during the last 30 minutes of each step. At this point, a steady-state is achieved whereby the glucose infused to maintain euglycaemia (5mmol/l) equilibrates to the total glucose disposal from the circulation.

Participants also attended a magnetic resonance imagining scan for assessment of visceral adipose tissue (VAT) area and intrahepatic lipid (IHL). VAT was assessed from the L4/5 anatomical position, and IHL was assessed as the average hepatic fat fraction from 8 circular regions of interests from the superior and inferior surface of the liver.

6.3.1 Calculations

Endogenous glucose production was assessed by measuring the glucose rate of appearance, expressed per m^2 of body surface area (BSA), during the basal period and the final 30 minutes of the low and high dose insulin infusion as discussed in chapter 2 (section 2.6.1). The hepatic insulin sensitivity index (HISI) was calculated as the reciprocal of the product of endogenous glucose production ($\mu mol/m^2 BSA\ min^{-1}$) and mean plasma insulin (pmol/l) (203, 344). This was used as an estimate of hepatic insulin sensitivity at basal and during insulin stimulation. The primary assessment of hepatic insulin sensitivity was calculated as the percentage suppression of endogenous glucose production from basal to the low dose insulin infusion steady state which accounts for differences in basal activity and represents an assessment of the dynamic change in glucose metabolism in response to the clamp (163, 204, 344). Finally, the residual endogenous glucose production during the steady-state of the high dose insulin infusion was assessed. Any negative or zeros values were defined as 100% suppression of endogenous glucose production, although this was minimised by enriching the glucose infusion with [6,6-$^2H_2$]-glucose to stabilise the tracer-to-tracee ratio.

6.3.2 Statistical analyses

Data were assessed for normality using the Shapiro-Wilks test and histograms. Normally distributed data are presented as mean (SD), data which required transformation (log10) are presented as geometric mean (95% CI) and data which remained skewed are presented as median (interquartile range). A two-way analysis of variance (ANOVA) was conducted to assess the main effect of ethnicity, glucose tolerance and the interaction. This was conducted on the normally distributed data and skewed data; however, the ethnicity findings for the latter were confirmed with Mann-Whitney U tests. To assess the

outcome of the two-way ANOVA, the interaction statistic was interpreted before interpreting the main effect of ethnicity or glucose tolerance. Where the interaction statistic was non-significant, the main effects of ethnicity and glucose tolerance were assessed independently. Glucose tolerance had more than two groups (NGT, IGT, T2D); thus, where a significant main effect was found, the Tukey's post hoc tests were employed to determine where the differences lay. To control for ethnic differences in VAT and IHL shown in chapter 3 (section 3.4.2); linear regression was used to assess the effect of ethnicity on insulin sensitivity whilst adjusting for glucose tolerance, VAT and IHL. These data are reported as the adjusted mean difference and 95% CI. Statistical significance was defined as $p < 0.05$. Data analyses were performed using SPSS software, version 25 (IBM Analytics, Armonk, NY, USA).

6.4 Results

The clinical characteristics of the participants assessed have been described in chapter 3. To summarise, all participants were adults aged between 18 to 65. BMI status identified most participants as either overweight or obese. There were no ethnic differences in BMI (p=0.98); however, there was a trend towards BAM being younger in age compared to WEM. There was a trend towards lower waist circumference in BAM compared to WEM (p=0.05), and the MRI analysis showed significantly lower VAT and IHL in BAM compared to WEM in each glucose tolerance group (p<0.01).

6.4.1 Endogenous glucose production

Endogenous glucose production (glucose Ra), which primarily reflects hepatic glucose production (129, 130), was assessed during the different clamp phases, as shown in table 5. During the basal state, there were no ethnic differences in endogenous glucose production in any glucose tolerance group (table 6). Basal endogenous glucose production appeared to be higher in WEM compared to BAM in participants with IGT or T2D however, this was not statistically significant. Basal endogenous glucose production was similar between NGT and IGT (p=0.45) however, participants with NGT or IGT had higher basal endogenous glucose production compared to those with T2D (p<0.01). Higher basal endogenous glucose production in participants with NGT and IGT was also found when expressing basal glucose production as a rate shown by a mean and standard deviation of NGT 917.8 (125.4) vs IGT 933.0 (158.6) vs T2D 803.5 (156.3) μmol/ min p<0.01.

During the steady-state of the low dose insulin infusion, there were no ethnic differences in endogenous glucose production in each glucose tolerance group (table 6). As expected, the endogenous glucose production during the low dose insulin infusion was

significantly lower in participants with NGT compared to T2D and IGT ($p<0.01$). There was a trend towards lower endogenous glucose production in participants with IGT compared to T2D ($p=0.06$).

6.4.2 Hepatic insulin sensitivity

The hepatic insulin sensitivity index (HISI) assesses endogenous glucose production whilst accounting for insulin. During the basal state, HISI showed no evidence for an ethnic difference in each glucose tolerance group (table 6, figure14A). The main effect of glucose tolerance on HISI showed a trend towards significance ($p=0.08$) such that participants with IGT had a lower HISI compared to participants with T2D ($p=0.05$) however, there were no other glucose tolerance differences.

During the low dose steady state, there were no ethnic differences in HISI in each glucose tolerance group (table 6). There was a statistically significant effect of glucose tolerance such that HISI was higher in NGT compared to IGT and T2D ($p<0.01$), but there was no difference between IGT and T2D ($p=0.95$).

The percentage suppression of endogenous glucose production from basal to the low dose insulin infusion steady-state was used as the primary assessment of hepatic insulin sensitivity (table 6, figure 14B). There was no statistical difference between BAM and WEM in any glucose tolerance group with a median ethnic difference of -8.4 vs 10.8 vs 9.6%, $p=0.62$ in men with NGT vs IGT vs T2D, respectively. Hepatic insulin sensitivity was significantly higher in participants with NGT compared with IGT and T2D ($p<0.01$). It was also significantly higher in participants with IGT compared to T2D ($p<0.01$).

Table 6: *Endogenous glucose production and hepatic insulin sensitivity before and during the hyperinsulinaemic-euglycaemic clamp*

	NGT		IGT		T2D				
	BAM 21	**WEM 23**	**BAM 10**	**WEM 9**	**BAM 18**	**WEM 15**	P_1 **Ethnicity**	P_2 **Glucose tolerance**	P_3 **Interaction**
Basal									
Endogenous glucose production; (µmol/m^{2}BSA min^{-1})[d]	444.9 (424.0, 466.8)	444.0 (424.7, 464.2)	417.9 (377.4, 462.8)	435.1 (407.0, 465.2)	369.0 (337.3,403.6)[a]	392.0 (352.1, 436.3)[a]	0.26	<0.01	0.59
Plasma insulin; pmol/l	51.7 (41.8, 64.0)[d]	47.2 (38.5, 58.0)[d]	68.8 (24.0)	81.6 (55.6)	44.5 (36.4, 54.4)[ad]	57.2 (39.2, 83.5)[ad]	NS	-	-
HISI; ((µmol /m^2 BSA min^{-1}) / (pmol/l))[d]	4.35 x10^{-5} (3.50, 5.40x10^{-5})	4.77 x10^{-5} (3.93, 5.78x10^{-5})	3.70 x10^{-5} (2.69, 5.08x10^{-5})	3.60 x10^{-5} (1.95, 6.64x10^{-5})	4.46 x10^{-5} (2.95, 6.74x10^{-5})[a]	6.09 x10^{-5} (4.90, 7.57x10-$^{-5}$)[a]	0.48	0.08	0.29

Continued

Table 6: *Endogenous glucose production and hepatic insulin sensitivity before and during the hyperinsulinaemic-euglycaemic clamp*

	NGT		IGT		T2D				
	BAM 21	**WEM 23**	**BAM 10**	**WEM 9**	**BAM 18**	**WEM 15**	**P_1 Ethnicity**	**P_2 Glucose tolerance**	**P_3 Interaction**
Low dose insulin infusion steady state									
Endogenous glucose production; ($\mu mol/m^2 BSA\ min^{-1}$)[e]	140.1 (127.3–187.8)[b]	105.3 (88.8–185.8)[c]	155.3 (133.6–260.2)	201.6 (174.7–285.6)	285.6 (175.0–294.6)	281.0 (217.1–360.3)	0.34	<0.01	0.21
HISI; (($\mu mol/m^2$ BSA min^{-1}) /(pmol/l))[e]	4.15×10^{-5} ($2.85–4.96 \times 10^{-5}$)[b]	5.19×10^{-5} ($3.43–6.99 \times 10^{-5}$)[b]	3.12×10^{-5} ($2.08–3.91 \times 10^{-5}$)	2.41×10^{-5} ($1.91–3.09 \times 10^{-5}$)	2.81×10^{-5} ($1.67–4.12 \times 10^{-5}$)	2.58×10^{-5} ($1.98–3.26 \times 10^{-5}$)	0.60	<0.01	0.79
Suppression of endogenous glucose production (%)[e]	66.1 (56.5 – 72.2)[b]	74.5 (55.9 – 80.2)[c]	60.5 (45.6 – 64.2)	49.7 (37.9 – 58.9)	40.4 (16.9 – 55.4)[a]	30.8 (16.5 – 56.8)[a]	0.62	<0.01	0.42

Continued

Table 6: *Endogenous glucose production and hepatic insulin sensitivity before and during the hyperinsulinaemic-euglycaemic clamp*

	NGT		IGT		T2D				
	BAM 21	**WEM 23**	**BAM 10**	**WEM 9**	**BAM 18**	**WEM 15**	P_1 **Ethnicity**	P_2 **Glucose tolerance**	P_3 **Interaction**
High dose insulin infusion steady state									
Endogenous glucose production; ($\mu mol/m^2 BSA\ min^{-1}$)	-14.3 (55.2)[b]	-13.8 (82.2)	37.3 (47.6)	36.4 (78.8)	51.1 (107.5)	76.6 (63.7)	0.62	<0.01	0.75

Data are expressed as mean (SD) for parametric data, geometric mean (95% CI)[d] for data which was parametric after log transformation and median (IQR)[e] for non-parametric data

P_1 : Main effect of ethnicity, P_2 : Main effect of glucose tolerance, P_3 : Ethnicity and glucose tolerance interaction

[a]sample size: BAM=15, WEM=12, [b]sample size =20, [c]sample size =21

Measurements were taken during the basal state and steady-state low and high dose insulin infusion. The percentage suppression from basal to the low dose insulin infusion steady state was used to represent hepatic insulin sensitivity.

Abbreviations: HISI- hepatic insulin sensitivity index, BSA- body surface area, NGT- normal glucose tolerance, IGT- impaired glucose tolerance, T2D- type 2 diabetes, BAM- black west African men, WEM- white European men, NS – nonsignificant

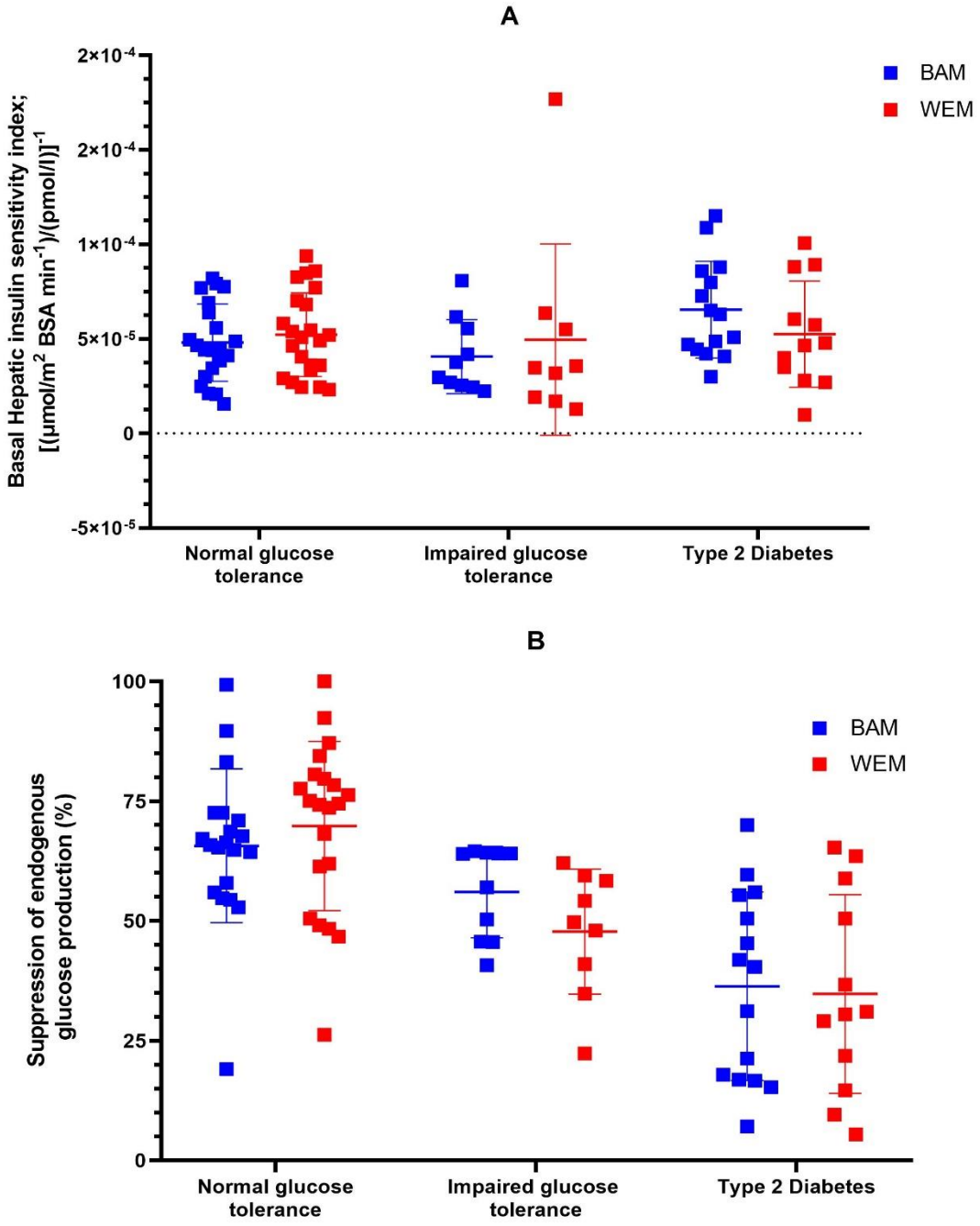


Figure 14: *Hepatic insulin sensitivity in black west African (BAM) and white European men (WEM) by glucose tolerance.*

Hepatic insulin sensitivity was calculated as A) endogenous glucose production as a function insulin (HISI) during the basal state, B) the percentage suppression of endogenous glucose production from the low dose insulin infusion steady-state compared to basal endogenous glucose production.

Data expressed as mean (SD).
Abbreviations: BSA – body surface area.

6.4.3 Hepatic insulin sensitivity adjustment for VAT and IHL

Based on the findings shown in chapter 3 (section 3.4.2) of lower visceral adipose tissue (VAT) and intrahepatic lipids (IHL) in BAM compared to WEM, the percentage suppression of endogenous glucose production was adjusted for VAT and IHL. The unadjusted mean difference and 95% CI in hepatic insulin sensitivity (the percentage suppression of endogenous glucose production) between BAM and WEM was 1.32 (-8.28, 10.92)%, p=0.79. Adjusting hepatic insulin sensitivity for VAT alone resulted in a trend towards lower hepatic insulin sensitivity in BAM compared to WEM. The overall adjusted mean difference and 95% CI was 6.49 (-0.82, 13.8)%, p=0.08. When adjusting hepatic insulin sensitivity for IHL alone, hepatic insulin sensitivity was lower in BAM compared to WEM although, this was not statistically significant with an overall mean difference and 95% CI of 5.10 (-2.26, 12.46)%, p=0.17. Combining VAT and IHL into the same regression model resulted in a trend towards lower hepatic insulin sensitivity in BAM compared to WEM with an overall adjusted mean difference and 95% CI of 7.36 (0.00, 14.73)%, p=0.05.

6.4.2 Endogenous glucose production during the high dose insulin infusion

The residual endogenous glucose production during the high dose insulin infusion steady-state is shown in table 5. There were no ethnic differences in endogenous glucose production across in any glucose tolerance group. There was a statistically significant effect of glucose tolerance on endogenous glucose production such that, participants with NGT had a significantly lower endogenous glucose production compared to those with IGT and T2D ($p<0.05$). There was no difference in endogenous glucose production between participants with IGT and T2D ($p=0.48$). Participants who achieved 100% suppression of endogenous glucose production during the high dose insulin infusion were higher in those with NGT (BAM=55%, WEM=48%), compared to those with IGT (BAM=20%, WEM=11%) or T2D (BAM=28%, WEM=7%). This was also true when assessing participants who achieved ≥90% suppression of endogenous glucose production show in figure 15.

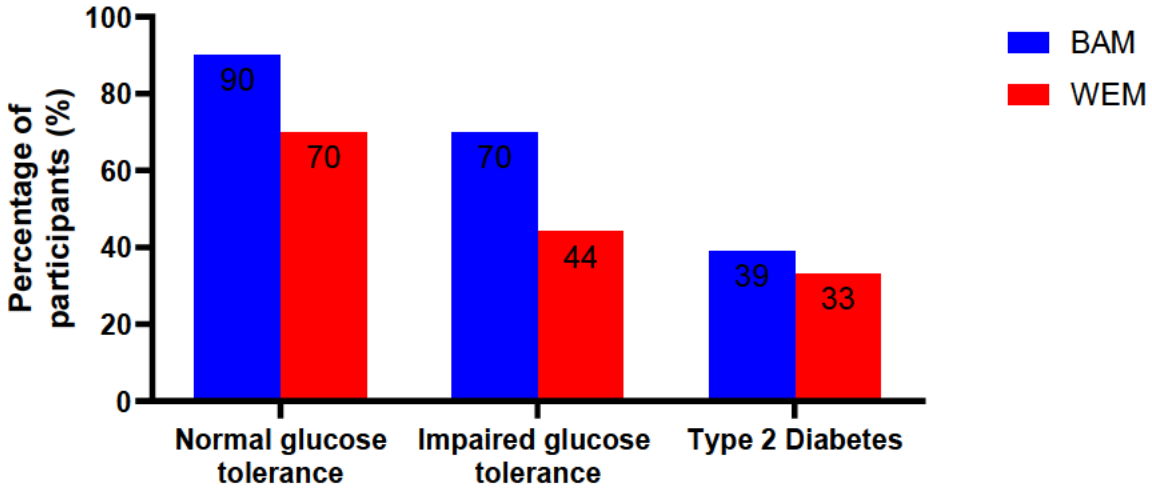


Figure 15: *The percentage of participants who achieved over 90% suppression of endogenous glucose production during the high dose insulin infusion of the clamp split by glucose tolerance and ethnicity.*

Abbreviations: BAM- black west African men, WEM- white European men

6.5 Discussion

The analysis in this chapter assesses the effect of ethnicity on hepatic insulin sensitivity measured from a two-step hyperinsulinaemic-euglycaemic clamp with a glucose isotopic tracer. There was no evidence for an ethnic difference in hepatic insulin sensitivity between black west African and white European men in any glucose tolerance group. This finding occurred when assessing hepatic insulin sensitivity during the basal and insulin stimulated state, this disagrees with the hypothesis for greater hepatic insulin sensitivity in black compared to white men.

There has been an abundance of reports which indicate that black communities are more insulin resistant in comparison to their white counterparts (256, 282-284). The methods utilised by these studies primarily assess peripheral insulin sensitivity. However, insulin is a pleiotropic hormone which acts at multiple sites across the body, including the liver (89, 90). In order to capture the hepatic response to insulin (the suppression of endogenous glucose production), isotopic tracers are required (159); tracers have not been utilised by the majority of the ethnicity-specific literature. Studies which have assessed hepatic insulin sensitivity in black and white participants have primarily been restricted to women and show inconsistent findings. This may be due to participant characteristics such as sex, glucose tolerance and obesity, or due to the method used to assess hepatic insulin sensitivity, i.e. basal compared to insulin stimulated assessments. This is the first analysis which has focused on black and white men over a range of glucose tolerances and BMI statuses which assesses both the basal and insulin-stimulated state. The finding of no ethnic difference in hepatic insulin sensitivity agrees with studies assessing men and women across a range of BMIs (315), lean women (322) and obese women (246). These studies have assessed insulin sensitivity using glucose tracers at baseline and

during insulin stimulation in participants with normal glucose tolerance or equal proportions of impaired glucose tolerance in black and white participants.

In contrast, the findings in this analysis do not align with *Goedecke et al.,* who studied severely obese women with an average BMI of 36.6 kg/m^2 and found greater hepatic insulin sensitivity in black compared to white women (323). *Goedecke et al.* assessed hepatic sensitivity using the same methodological procedure and hepatic insulin sensitivity calculations as this study; thus, the differences in findings are not likely to be due to methodology. A comparison of the median percentage suppression of endogenous glucose production reported in this study and *Goedecke et al.,* show that the black men with normal glucose tolerance in this study had 15% higher hepatic insulin sensitivity than black women studied by *Goedecke et al.* However, the white men studied in this analysis had 77% higher hepatic insulin sensitivity than white women studied by *Goedecke et al.* Therefore, the disagreement between ethnic differences in this analysis and *Goedecke et al.* may reflect the observation that the white women studied were significantly more resistant than the white men studied in this analysis. Whilst there is no cut-point to define hepatic insulin resistance, the median percentage suppression of endogenous glucose production for the white women reported by *Goedecke et al.* was also lower than studies in healthy participants, NAFLD and T2D patients using the same insulin clamp dose (163, 376). This adds further evidence to the notion that the white women studied by *Goedecke et al.* may have exhibited pronounced insulin resistance. Therefore, it could be interpreted that the black women did not have greater hepatic insulin sensitivity but rather the control white women were more insulin resistant than expected.

The contrasting results between this study and *Goedecke et al.* may also reflect a combined sex and obesity effect such that ethnic differences are present in severe obesity

in women but absent for men. In support of obesity status potentially influencing ethnic differences in hepatic insulin sensitivity, a study which assessed lean women found no ethnic difference in basal or stimulated hepatic insulin sensitivity (322). In comparison, an assessment of severely obese women using a similar protocol did find an ethnic difference (323) which suggests that ethnic differences in hepatic insulin sensitivity are more apparent in the presence of obesity. In support of sex altering ethnic differences, an assessment of whole-body insulin sensitivity in black South African men and women has shown men to be more insulin sensitive (353). The higher insulin sensitivity in black men may be close to the insulin sensitivity values displayed by white men which may explain why no ethnic differences were identified in the men in this study. Whilst a sex and obesity influence may be interpreted from these findings and the literature, data comparing black and white women do not fully support this sex and obesity interaction. A more recent study in American obese black and white women, with an average BMI of 32.5kg/m^2, has assessed basal hepatic insulin sensitivity and found no ethnic difference (246) whereas south African obese black women are shown to have greater hepatic insulin sensitivity (323). This undermines the suggestion of ethnic differences being present in obese women and suggests assessments of geographical influences are warranted and suggests that grouping participants as 'black' may be inappropriate. In further support of geographical differences influencing ethnicity findings, South African black women appear to display a distinct diabetogenic phenotype shown by the higher prevalence of obesity and T2D in black South African women compared to black African men residing in the UK (259, 377).

The findings in this chapter also conflict with *Ellis et al.*, who reported lower basal and stimulated hepatic insulin sensitivity in normal glucose tolerant black compared to white women across a range of BMIs (296). Their insulin-stimulated finding was based

on a mathematical model with the intravenous glucose tolerance test and glucose tracer, which has yet to be validated (322), thus cannot be directly compared to the findings in this analysis. However, their assessment of basal hepatic insulin sensitivity is comparable to the method used in this analysis (296). *Ellis et al.* found lower basal hepatic insulin sensitivity in black women (296) which contrasts with the analysis of men in this chapter and may provide further evidence for a sex difference in the effect of black ethnicity on hepatic insulin sensitivity. In contrast to the findings of studies in women which show inconsistent results, the findings in this chapter show a consistent message in men regardless of their adiposity status, glucose tolerance or method for assessing hepatic insulin sensitivity. In each glucose tolerance group, hepatic insulin sensitivity was similar in white and black men which could imply that pronounced hepatic insulin resistance does not explain the increased risk of T2D in black communities. It may also imply that the progression of hepatic insulin resistance with impaired glucose tolerance is not ethnically different. In contrast, the conflicting ethnic-specific results in the literature may reflect an interaction between sex, obesity and geographical region which impact whether ethnic differences in hepatic insulin sensitivity are present. Ethnic differences appear to be present more so in women; whether it is higher or lower in black women depend on obesity and geographical region of the black women; however, from this analysis, ethnic differences are absent in black and white men.

The presence of high visceral adipose tissue (VAT) and intrahepatic lipids (IHL) has been significantly associated with hepatic insulin resistance (203-205, 226, 373). The characteristics presented in chapter 3 (section 3.4.2) showed significantly lower VAT and IHL in the black men which agree with the literature (247, 256). Thus, similar hepatic insulin sensitivity occurred in the presence of lower visceral and hepatic fat in black men. When adjusting hepatic insulin sensitivity for VAT and IHL, black men presented with a

non-significant trend towards lower hepatic insulin sensitivity. This may suggest that having lower levels of visceral and hepatic fat is protective in black men and prevents them from presenting with greater hepatic insulin resistance. Whilst IHL is consistently reported to be lower in black populations, a study which assessed black and white patients with fatty liver disease showed no ethnic difference in estimated insulin resistance (326). This may indicate that other variables outside of IHL are protecting black populations from having pronounced hepatic insulin resistance.

The analysis in this chapter was derived from data from the two-step hyperinsulinaemic-euglycaemic clamp. Hepatic insulin sensitivity was assessed during the low dose insulin infusion, which was below the maximum concentration for complete suppression of endogenous glucose production in all glucose tolerances. The higher dose insulin infusion was used to assess peripheral glucose disposal at which point the method assumed complete suppression of hepatic glucose production (156). The residual endogenous glucose production during the high dose infusion has also presented in this chapter. There were no ethnic differences in residual endogenous glucose production which further suggest similar hepatic insulin sensitivity at this high dose. However, endogenous glucose production increased with worsening glucose tolerance reflecting the increasing hepatic insulin resistance, which was also observed during the low dose insulin infusion. Accounting for the residual endogenous glucose production is of importance when drawing any conclusions during the high dose infusion and this discussed in more detail in chapter 9.

Whilst this chapter did not aim to assess the effect of glucose tolerance on hepatic insulin sensitivity, the finding of significantly lower endogenous glucose production in participants with T2D compared to those without T2D was unexpected. Increased hepatic glucose production is commonly shown in T2D (132, 378, 379). Expressing the glucose

production as a rate (μmol/ min) to remove the metabolic body size parameter (BSA) did not change the significant impact of glucose tolerance. Impairments in hepatic glucose production have been described to be more pronounced in severe T2D when compared to the early stages of T2D (380, 381) which may explain why the participants with early type 2 diabetes did not have higher basal EGP. This is also supported by studies which show no difference in basal endogenous glucose production between Swedish men of NGT, IGT and mild T2D (129) or between lean and obese participants (382). Adjusting for insulin via the hepatic insulin sensitivity index reduced the glucose tolerance effect. However, there was a trend towards greater hepatic insulin sensitivity in participants with T2D compared to IGT. The apparent greater hepatic insulin sensitivity in T2D may be due to residual effects of the metformin. The majority of the participants were on metformin, and this has been shown to reduce hepatic insulin resistance in T2D independently of weight change (383). However, participants were instructed to stop medication 7 days before their metabolic assessments to reduce this effect; therefore, this explanation is less likely. A more likely reason for lower endogenous glucose production in participants with T2D is that the participants with NGT were less insulin resistant and therefore, needed to make more glucose to maintain plasma glucose following a prolonged fast. This is supported by the peripheral glucose disposal data which showed lower insulin resistance in NGT. It may also be the result of a lack of statistical power as the sample size in T2D was not large. Overall, the hepatic insulin response finding (percentage suppression of endogenous glucose production) does agree with the consensus that hepatic insulin resistance increases as glucose tolerance gets worse. Therefore this data shows, the main effect of glucose tolerance on hepatic insulin sensitivity differs depending on the assessment of hepatic insulin sensitivity.

To conclude, hepatic insulin sensitivity appears to be similar between black west African and white European men across a range of glucose tolerances which disagrees with the hypothesis of greater hepatic insulin sensitivity in black compared to white men. This finding occurs when expressing hepatic insulin sensitivity in multiple ways in participants with a range of BMIs. The comparable hepatic insulin sensitivity occurs in the presence of lower visceral and hepatic fat in black men. Lower storage of visceral and hepatic fat may protect black men from having greater hepatic insulin resistance.

Chapter 7: A comparison of adipose tissue insulin sensitivity between black and white men

Chapter highlights:

- There were no ethnic differences in adipose insulin sensitivity in all glucose tolerance groups.
- Absolute rates of lipolysis were significantly lower in BAM, this was not explained by insulin but could partially be explained by lower visceral fat in BAM.
- Lower rates of lipolysis in BAM occurred in the presence of lower triglycerides and may contribute towards ethnic differences in plasma triglycerides.

Data presented in this chapter have been published:

Bello O, Mohandas C, Shojaee-Moradie F, Jackson N, Hakim O, Alberti KGMM, Peacock JL, Umpleby AM, Amiel SA, Goff LM. Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat. Diabetologia. 2019 May;62(5):835-844.

Bello O, Ladwa M, Hakim O, Marathe C, Shojaee-Moradie F, Charles-Edwards G, Peacock JL, Umpleby MA, Amiel S, Goff LM. Differences in the link between insulin sensitivity and ectopic fat in men of Black African and White European ethnicity. Eur J Endocrinol. 2019 Nov;182 (1):91-101

7.1 Introduction

Lipolysis is the process by which triglyceride molecules are hydrolysed into free fatty acids and glycerol. This occurs in multiple tissues; however, adipose tissue is the only one which releases the fatty acids and glycerol for use in non-adipose tissue (138). Insulin has been well characterised as a potent inhibitor of adipose tissue lipolysis (72, 100, 107, 139-142). Resistance to insulin-mediated suppression of lipolysis has been implicated in the development of type 2 diabetes (T2D) (152-154, 384). Adipose tissue insulin resistance has been shown to be up to 4 times higher in T2D compared to normal glucose tolerance (153, 154).The insulin resistance to lipolysis results in inappropriately high rates of lipolysis causing elevated circulating fatty acids, which promote peripheral and hepatic insulin resistance through lipotoxicity and by increasing gluconeogenesis (107, 128, 135, 136, 221, 233, 241). Lipolysis occurs in both subcutaneous and visceral adipose tissue depots; the latter has been described as a highly lipolytic fat store (211). Stable isotopic tracers have been employed in the assessment of lipid kinetics whereby the flux of glycerol appearance into the circulation is used as a direct quantification of lipolysis. Particularly during the fasted state, fatty acid and glycerol entry into the circulation primarily reflects lipolysis in peripheral adipose tissue. Lipolysis also occurs in the circulation as triglycerides in lipoproteins are hydrolysed; however, this has a minor contribution to total lipolysis as shown by relatively low VLDL clearance rates (143, 144, 241). There is currently no “gold standard” method to assess adipose tissue insulin sensitivity to lipolysis; however, using isotopic tracers in the presence of insulin has been highly regarded (166, 384, 385).

Only a single *in vivo* study has assessed the effect of insulin on adipose tissue lipolysis using tracers in black and white adults (304). *Albu et al.* showed significantly greater insulin-mediated suppression of lipolysis in obese black women compared to

white women who were matched for BMI which was 34.9kg/m^2 on average (304). In comparison, a recent study of obese black and white women who were also matched for BMI (32.5kg/m^2 on average) found no ethnic difference in basal adipose tissue insulin sensitivity (246). Other studies have used isotopic tracers to assess lipolysis, without accounting for insulin, and have shown either no ethnic difference in lipolysis (303, 386) or lower lipolysis (252, 304, 325, 387) in black compared to white populations. These studies have predominantly been conducted in obese women; however, whether they can be extrapolated to men is unknown. Sex differences in adipose tissue function, outside of ethnicity, have been reported such that women display higher rates of lipolysis in comparison to men (388, 389) suggesting that findings in women may not be extrapolated to men. Whether the ethnic findings differ depending on glucose tolerance is also unknown.

7.2 Aim

This chapter aims to determine if there are ethnic differences in the effect of insulin on adipose tissue lipolysis between black west African (BAM) and white European men (WEM) of normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or early type 2 diabetes (T2D).

It could be hypothesised that BAM will display greater adipose tissue insulin sensitivity compared to WEM.

7.3 Methods

Chapter 2 provides a full description of the study methods which include the participant inclusion criteria, metabolic assessments and the calculations used to derive measures of insulin sensitivity. In brief, black west African (BAM) and white European men (WEM) were defined as normal glucose tolerant (NGT) or impaired glucose tolerant (IGT) based on an oral glucose tolerance test. Participants defined as having type 2 diabetes (T2D) had been diagnosed by their primary care practitioner within 5 years.

Eligible participants were invited to attend a two-step hyperinsulinaemic-euglycaemic clamp with a stable [$^2H_5$]-glycerol isotope infusion to assess whole-body / total glycerol rate of appearance (Ra). Whole-body / total glycerol Ra primarily reflects adipose tissue lipolysis, particularly during the basal state; although the hydrolysis of circulating triglyceride-rich lipoprotein contribute a small portion of glycerol Ra which increases during insulin stimulation (143, 234). In this study, whole-body/total glycerol Ra is referred to as adipose tissue lipolysis. This was assessed during the basal phase and low dose insulin infusion to calculate adipose tissue insulin sensitivity. To begin the procedure, a primed continuous infusion of [$^2H_5$]-glycerol was initiated at time -120 minutes and ran until time 120 minutes. The assessment of basal adipose tissue lipolysis was made from samples drawn from time -30 to 0 minutes; by which time the [$^2H_5$]-glycerol glucose isotope infusion had reached an equilibrium enrichment with plasma glycerol. At time 0 minutes, an insulin infusion was initiated at 10mU/m^{2}BSA/min for 120 minutes, which defined the first step of the hyperinsulinaemic-euglycaemic clamp, termed the low dose insulin infusion. Adipose tissue lipolysis during the low dose insulin infusion was assessed from samples drawn during the last 30 minutes. At this point, a steady-state was achieved whereby the glucose infused to maintain euglycemia (5mmol/l) equilibrated to the total glucose disposal from the circulation.

Participants also attended a magnetic resonance imagining scan for assessment of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) area at the L4/5 anatomical position.

7.3.1 Calculations

The rate of adipose tissue lipolysis was determined by measuring the glycerol rate of appearance, expressed per m^2 of body surface area (BSA), during the final 30 minutes of the basal and low dose insulin stimulation. The primary assessment of adipose tissue insulin sensitivity was calculated as the percentage suppression of lipolysis from basal to the low dose insulin stimulation which accounts for differences in basal activity and reflects the response to the insulin clamp (166, 224, 343). The adipose tissue insulin sensitivity index (ATIS) was also calculated as the reciprocal of the product of adipose tissue lipolysis ($\mu mol/m^2 BSA\ min^{-1}$) and mean plasma insulin (pmol/l) (153, 165, 166). This was used as an estimate of adipose tissue insulin sensitivity at basal and during insulin stimulation.

7.3.2 Statistical analyses

The Shapiro-Wilks test and histograms were used to assess the distribution of data for each dependent variable. Data which were normally distributed are presented as mean (SD), and data which required transformation (log10) are presented as geometric mean (95% CI). To assess the main effect of ethnicity, glucose tolerance and the interaction, a two-way analysis of variance (ANOVA) was conducted on the normally distributed data. For the dependent variable which required a transformation (log 10) in two glucose tolerance groups but not the other, the transformed (log 10) data were used in the two-way ANOVA, and the ethnicity finding was confirmed with Mann-Whitney U tests. To

assess the outcome of the two-way ANOVA, the interaction statistic was interpreted before interpreting the main effect of ethnicity or glucose tolerance. Where the interaction statistic was not significant, the main effect of ethnicity and glucose tolerance could be assessed in isolation. Glucose tolerance had more than two groups (NGT, IGT, T2D); thus, where a significant main effect was found, the Tukey's post hoc tests were employed to determine where the differences lay. To control for ethnic differences in VAT and IHL shown in chapter 3 (section 3.4.2), linear regression was used to assess the effect of ethnicity on insulin sensitivity whilst adjusting for glucose tolerance and VAT. These data are reported as the unadjusted and the adjusted mean difference and 95% CI for normally distributed data and the adjusted and unadjusted ratio of the geometric mean and 95% CI for log-transformed data.

To comply with statistical test assumptions of an ANOVA, in cases where the variance of the characteristic was unequal among groups (assessed using the Levene's statistic), a more stringent statistical significance cut-off has been employed at $p<0.01$. Otherwise, statistical significance was defined as $p<0.05$. Data analyses were performed using SPSS software, version 25 (IBM Analytics, Armonk, NY, USA).

7.4 Results

The characteristics of the participants assessed in this analysis have been described in detail in chapter 3. To summarise, BAM and WEM were similar in BMI, by design, with most participants identified as overweight or obese. Participants with IGT and T2D were more overweight or obese than those with NGT. There was a trend towards an ethnic difference in age such that, BAM were younger than WEM, specifically those with IGT ($p=0.02$); however, all participants were between the age of 18 and 65. In all glucose tolerance groups, BAM displayed significantly lower VAT than WEM ($p<0.01$).

7.4.1 Adipose tissue lipolysis

Adipose tissue lipolysis, measured as the rate of glycerol appearance, was assessed during the basal and low dose insulin steady-state period of the hyperinsulinaemic-euglycaemic clamp, shown in table 6. During the basal state, BAM exhibited significantly lower lipolysis compared to WEM in all glucose tolerance groups (table 7). The mean ethnic difference was most pronounced in participants with IGT. Similar to the basal state, BAM presented with significantly lower adipose tissue lipolysis during the low dose insulin steady state of the clamp, compared to WEM. This was apparent in all glucose tolerance groups (table 7). During both the basal and low dose insulin infusion steady-state, glucose tolerance status had a significant effect on adipose tissue lipolysis such that lipolysis was significantly greater in participants with T2D compared to NGT or IGT ($p<0.01$). However, there were no differences between participants with NGT or IGT ($p \geq 0.77$).

7.4.2 Insulin sensitivity to adipose tissue lipolysis

Adipose tissue lipolysis was adjusted for insulin using the adipose tissue insulin sensitivity index (ATIS); basal ATIS was higher in BAM compared to WEM (table 7, figure16A). During the low dose insulin infusion steady-state, BAM presented with a non-significant trend towards higher ATIS compared to WEM (table 6). This finding was based on a more stringent significance cut-off to account for the unequal variances between groups (see section 7.3.2).

The percentage suppression of adipose tissue lipolysis from basal to the low dose insulin infusion steady state was used as the primary assessment of adipose tissue insulin sensitivity because it accounts for the ethnic differences in basal lipolysis activity and is a response to insulin (table 7, figure16B). There was no statistical difference in percentage lipolysis suppression between BAM and WEM in all glucose tolerance groups. The mean ethnic difference was -8.5 vs 0.2 vs-0.3 % in men with NGT, IGT and T2D, respectively.

For the various measures of adipose tissue insulin sensitivity which showed a statistically significant effect of glucose tolerance, participants with NGT were more insulin sensitive compared to those with T2D ($p=0.02$) whereas there was no statistical difference between those with NGT and IGT or IGT and T2D ($p>0.28$).

Table 7: Adipose tissue lipolysis and adipose tissue insulin sensitivity before and during the hyperinsulinaemic-euglycaemic clamp

	NGT		IGT		T2D				
	BAM 20	**WEM 23**	**BAM 10**	**WEM 9**	**BAM 18**	**WEM 15**	P_1 Ethnicity	P_2 Glucose tolerance	P_3 Interaction
Basal									
Adipose tissue lipolysis; (μmol/m^{2}BSA min^{-1})[a]	42.7 (31.2,58.4)	56.5 (46.8, 68.2)	36.9 (30.1, 45.2)	60.0 (50.8, 70.7)	64.1 (55.0, 74.7)[c]	78.4 (66.6, 92.2)[d]	<0.01	<0.01	0.54
ATIS; [(μmol /m^2 BSA min^{-1})*(pmol/l)]$^{-1}$	4.48 x10^{-4} (3.18,6.31x10^{-4})[a]	3.75 x10^{-4} (2.79,5.03x10^{-4})[a]	4.18 x10^{-4} (3.19,5.48x10^{-4})[a]	2.61 x10^{-4} (1.32,5.15x10^{-4})[a]	3.82 x10^{-4} (1.54 x10^{-4})[c]	2.68 x10^{-4} (1.37 x10^{-4})[d]	0.02	0.07	0.72
Low dose insulin infusion steady state									
Adipose tissue lipolysis; (μmol/m^{2}BSA min^{-1})[a]	20.8 (17.1, 25.3)	24.9 (20.45, 30.4)[b]	19.5 (16.0, 23.6)	32.2 (24.2, 42.8)	41.3 (33.3, 51.2)	49.4 (41.0, 59.5)[e]	<0.01	<0.01	0.29
ATIS; [(μmol /m^2 BSA min^{-1})*(pmol/l)]$^{-1}$	3.11 x10^{-4} (1.40 x10^{-4})	2.79 x10^{-4} (1.39 x10^{-4})[b]	2.91 x10^{-4} (8.36 x10^{-5})	1.92 x10^{-4} (9.12 x10^{-5})	1.73 x10^{-4} (8.03 x10^{-5})	1.41 x10^{-4} (4.26 x10^{-5})[e]	0.03	<0.01	0.50
Suppression of adipose tissue lipolysis (%)	45.3 (25.3)	53.8 (17.4)[b]	45.7 (13.7)	45.5 (9.8)	37.2 (16.0)[c]	37.5 (13.7)[f]	0.49	0.04	0.56

Data are expressed as mean (SD) for parametric data or geometric mean (95% CI)[a] for data which was parametric after log transformation.
P_1 : Main effect of ethnicity, P_2 : Main effect of glucose tolerance, P_3 : Ethnicity and glucose tolerance interaction
[b]Sample size: WEM=22, [c]Sample size: BAM=15, [d]Sample size: WEM=12, [e]Sample size: WEM=13, [f]Sample size: WEM=10
The percentage suppression of adipose tissue lipolysis was assessed from the basal to and low dose insulin infusion steady state.
Abbreviations: ATIS- adipose tissue insulin sensitivity index, BSA- body surface area, NGT- normal glucose tolerance, IGT- impaired glucose tolerance, T2D- type 2 diabetes, BAM- black west African men, WEM- white European men.

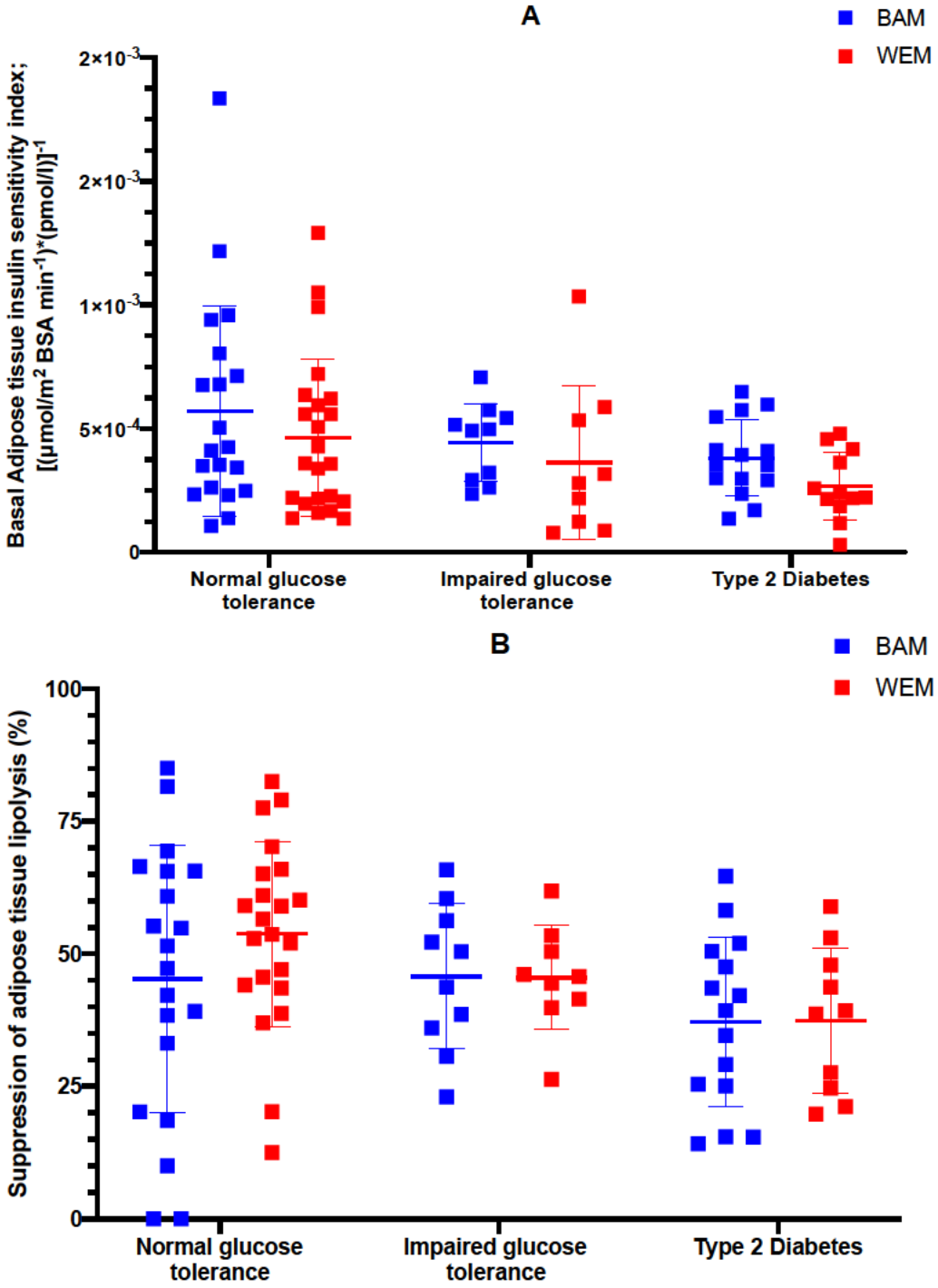


Figure 16: *Adipose tissue insulin sensitivity in black west African (BAM) and white European men (WEM) by glucose tolerance.*

Adipose tissue lipolysis insulin sensitivity during the basal state using the adipose tissue insulin sensitivity index (A) and when assessed as the suppression of adipose tissue lipolysis from basal to low dose insulin infusion, 10 mU/m²BSA/min, during a hyperinsulinaemic-euglycaemic clamp (B).

Data expressed as mean (SD). Abbreviations: BSA- body surface area.

7.4.3 Adjusting lipolysis for VAT and SAT

The findings in chapter 3 (section 3.4.2) showed lower VAT in BAM compared to WEM but no ethnic difference in SAT.

The overall unadjusted ratio of the geometric mean and 95% CI for basal ATIS was 1.35 (1.01, 1.80), p=0.045 which reflects higher basal ATIS in BAM compared to WEM. After adjusting basal ATIS for VAT, the ethnic difference was lost (ratio of the geometric mean and 95% CI of 1.16 (0.87, 1.54) p=0.31). When adjusting basal ATIS for SAT, the ethnic difference in geometric mean and 95%CI remained significant at 1.36 (1.03, 1.80), p=0.029. Adjusting basal ATIS for VAT and SAT resulted in no ethnic difference with a ratio of the geometric mean and 95% CI of 1.19 (0.89, 1.61), p=0.24.

ATIS was also assessed during the low dose insulin infusion steady state with an overall unadjusted mean difference and 95% CI of 3.47 $x10^{-5}$ (-1.8 $x10^{-5}$, 8.8 $x10^{-5}$) [(μmol /m^2 BSA min^{-1})*(pmol/l)]$^{-1}$, p=0.20 between BAM and WEM. Adjusting ATIS during the low dose insulin infusion for VAT resulted in an adjusted mean difference and 95% CI of 1.39 $x10^{-5}$ (-3.4 $x10^{-5}$, 6.1 $x10^{-5}$) [(μmol /m^2 BSA min^{-1})*(pmol/l)]$^{-1}$, p=0.56. Adjusting ATIS calculated during the low dose insulin infusion for SAT resulted in a mean difference and 95% CI of 3.76 $x10^{-5}$ (-6.0 $x10^{-6}$, 8.2 $x10^{-5}$) [(μmol /m^2 BSA min^{-1})*(pmol/l)]$^{-1}$, p=0.09. Adjusting ATIS during the low dose insulin infusion for VAT and SAT resulted in no ethnic difference with an adjusted mean difference and 95% CI of 2.93 $x10^{-5}$ (-1.9 $x10^{-5}$, 7.7 $x10^{-5}$) [(μmol /m^2 BSA min^{-1})*(pmol/l)]$^{-1}$, p=0.23.

7.5 Discussion

The analyses in this chapter assessed the effect of ethnicity on insulin-mediated suppression of lipolysis (adipose tissue insulin sensitivity) in black west African and white European men. Adipose tissue insulin sensitivity was assessed during a hyperinsulinaemic-euglycaemic clamp with a stable glycerol isotopic tracer. The findings suggested that there were no ethnic differences in the adipose tissue insulin sensitivity to lipolysis, which disagrees with the hypothesis of greater adipose tissue insulin sensitivity in black men. This finding was consistent in each glucose tolerance group.

Populations of black ethnicity are at high risk of developing T2D and are repeatedly reported to be more insulin resistant as shown by multiple methods (256, 265, 282-286) (256, 282-284). This view primarily focuses on insulin-mediated effects on glucose homeostasis. Insulin has also been shown to have a direct influence on lipid kinetics such that it reduces adipose tissue lipolysis; resistance to this action is described as an early defect in the progression to T2D (152-154, 384). Studies in black and white populations which assess changes in lipolysis or lipid kinetics in response to insulin are scarce. Prior to this analysis, no *in vivo* study had focused on insulin-mediated suppression of lipolysis in black and white men. Previous studies have been restricted to obese women who show either greater adipose tissue insulin sensitivity in black compared to white women (304) or no ethnic difference in adipose tissue insulin sensitivity (246). The conflicting results may reflect differences in the method used to assess adipose tissue insulin sensitivity in obese women. In one study, changes in lipolysis were assessed in response to an insulin stimulation (304), whereas the other study assessed basal adipose tissue insulin sensitivity (246). The different findings in obese women may also reflect differences in VAT content which is of importance because

VAT is a highly lipolytic fat store, and high VAT is an indicator of metabolic dysfunction (211, 324). This analysis provides evidence that the suppression of lipolysis in response to insulin is similar in black and white men across all glucose tolerance groups, suggesting that there are no ethnic differences in adipose tissue insulin sensitivity between black and white men. The primary assessment of the insulin-mediated effect on adipose tissue lipolysis was the percentage change in lipolysis from basal to the low dose insulin infusion steady-state. This calculation accounts for differences in basal activity, hence, reflects the change in lipolysis in response to the insulin infusion applied. However, the black men began with lower basal lipolysis and continued to have lower lipolysis during the insulin stimulation. The percentage decrease in lipolysis was similar in black and white men, particularly in those with IGT or T2D. Therefore, it can be concluded that the insulin-stimulated response was similar in black and white men.

In comparison to the literature, the finding of no ethnic difference in adipose tissue insulin sensitivity disagrees with *Albu et al*., who found greater insulin-mediated suppression of lipolysis in black compared to white obese women with an overall average BMI of 34.9 kg/m^2 (304). The different sexs of the participants studied here and by Albu et al. may explain why their findings disagree with the analyses in this chapter. It has been reported that lipolysis is greater in women compared to men; hence the glycerol and fatty acid flux is higher (388-390). It could be postulated that an ethnic difference in insulin-mediated suppression of lipolysis may not be present in men because basal lipolysis is at a comparatively low rate compared to women, and to see an ethnic difference may require a higher baseline value. Differences in the methodology may also contribute an explanation as to why *Albu et al.,* found a difference which was not present in this study of men. This

analysis utilised the hyperinsulinaemic-euglycaemic clamp (156) whereas *Albu et al.* utilised a pancreatic clamp. Whilst the hyperinsulinaemic-euglycaemic clamp has been termed the "gold standard" assessment of insulin sensitivity, this view focuses on insulin-mediated glucose uptake. There is currently no agreement for the "gold standard" procedure or calculation to assess the antilipolytic action of insulin (384, 385). It is accepted that isotopic tracers work well at tracing lipid metabolism, particularly glycerol as it is less actively re-esterified back into triglycerides, which would lead to an underestimation of lipolysis (385). However, an optimal metabolic procedure in which to apply the tracers has yet to be concluded. A number of studies have calculated the percentage suppression of lipid appearance in response to insulin (162, 165, 166) however; there is no consensus on the ideal equations for adipose tissue insulin sensitivity assessments (151, 166, 385). Currently, a multistep pancreatic clamp using somatostatin and low doses of insulin has been proposed as the most sensitive procedure to assess insulin's antilipolytic action in the adipose tissue (385). Somatostatin, used in a pancreatic clamp, suppresses endogenous insulin and allows for a measurement of lipolysis at an insulin concentration of zero. Insulin doses, chosen by the investigator, can then be applied during the multistep clamp to assess the change in lipid flux. From this procedure, the IC50 is calculated and defined as the insulin concentration required to produce half the maximal response. A comparison of the IC50 from a single step hyperinsulinaemic-euglycaemic clamp and multistep pancreatic clamp has shown a significant association (151); thus the hyperinsulinaemic-euglycaemic clamp is also a robust method to assess insulin's antilipolytic activity. This may imply that although different methodological procedures were used by *Albu et al.* in comparison to this study, both procedures produce findings which correlate well with one another. Differences in the

procedure to assess adipose tissue insulin sensitivity are not likely to explain the difference in findings. To date, *Albu et al.* is the only study to utilise a pancreatic clamp to assess and compare the lipolysis response to insulin in black and white adults however, lipolysis at zero insulin was not measured and the IC50 was not interpreted. They did use lower insulin doses compared to this study (2 mU/m^2BSA/min and 8 mU/m^2BSA/min), which also form part of the methodological difference and could be involved in the contrasting findings. Overall, sex and obesity differences are likely to explain why ethnic differences were not found in men but were apparent in obese women. Interestingly, the sex differences explanation is not conclusive based on the literature. *Chung et al.* recently studied obese black and white women, similar to *Albu et al.*, with an average BMI of 32.5kg/m^2 and reported no ethnic difference in basal adipose tissue insulin sensitivity (246). This suggests that the result of an ethnic comparison between adipose tissue insulin sensitivity in women alone also depend on whether inulin sensitivity is assessed during the basal or insulin stimulated state. The present study shows no impact of ethnicity on adipose tissue insulin sensitivity when assessed during a clamp and contributes to the literature by showing no ethnic differences in men in any glucose tolerant group. The data imply the pronounced adipose tissue insulin resistance is not likely to contribute to the increased risk of T2D in black communities.

The analysis in this chapter is focused on the insulin-mediated actions on lipolysis. The percentage suppression of lipolysis from basal to the low dose insulin infusion was the primary assessment of adipose tissue insulin sensitivity, and there was no evidence of an ethnic difference. However, the analysis did show that black men have lower absolute rates of lipolysis at baseline and during the clamp when assessed in isolation. When accounting for insulin, using ATIS, the ethnic difference remained, which may suggest that black men

have greater adipose tissue insulin. Lower rates of lipolysis have been reported almost consistently in black populations (252, 304, 325, 387) although a few groups have shown no difference (303, 386). This may suggest that black individuals have an intrinsic push towards lower circulating free fatty acids or resistance to high circulating free fatty acids. Some studies have attributed the lower rates of lipolysis in black individuals to lower levels and gene expression of hormones known to regulate adipose tissue lipolysis. Hormone-sensitive lipase (391) and adipose triglyceride lipase (392) have been reported to be lower in black compared to white women. These enzymes hydrolyse triglycerides and are therefore mediators of lipolysis, so lower quantities of these enzymes may explain lower rates of lipolysis.

Triglycerides are hydrophobic molecules; therefore, in the aqueous plasma, the primary sources of triglycerides are within lipoprotein complexes, e.g. chylomicrons which contain dietary lipids or VLDLs which are produced by the liver (393). The latter is the greatest source of triglycerides during the postabsorptive state. The endogenous production of VLDLs by the liver involves fatty acids and triglycerides from multiple sources including chylomicron remnants which remain after Lipoprotein lipase hydrolysis of chylomicrons; *de novo* lipogenesis in response to hyperglycaemia and hyperinsulinaemia; triglycerides stored in the liver (intrahepatic lipids) and free fatty acids in the plasma pool bound to albumin which is predominantly from lipolysis of triglycerides in adipose tissue (394). Due to the latter, adipose tissue lipolysis has been shown to associate with circulating triglycerides, the product of lipolysis (fatty acids) are taken up by the liver as a precursor/substrate molecule for triglyceride-rich VLDL synthesis (142, 241, 395-397). The relatively low rate of lipolysis in the presence of lower triglyceride concentrations observed in the black men may reflect

this association. The low rates of lipolysis may contribute towards the low plasma triglycerides in black compared to white individuals.

Visceral adipose tissue (VAT) has been shown to be a highly lipolytic fat depot (211). The black men analysed in this chapter had lower VAT compared to the white men, which agrees with the existing literature (398, 399). Accounting for insulin did not explain the ethnic differences in lipolysis at baseline or during insulin stimulation which agrees with a study in black and white adolescents (400). However, after adjusting lipolysis for VAT, the ethnic differences in lipolysis were lost. This may suggest that lower VAT contributes towards the lower lipolysis in black men. In comparison, adjusting lipolysis for SAT, which was not ethnically different, did not change whether a statistical difference was observed between black and white men. This may suggest that the volume of SAT is not contributing to the lower rates of lipolysis in black men. This analysis is limited in that the contribution of lipolysis from visceral and subcutaneous fat stores cannot be determined, whole-body/total lipolysis was assessed rather than tissue-specific lipolysis. In addition, it is not clear whether the lower VAT is causing lower overall systemic lipolysis or if the lower rates of lipolysis are leading to less VAT accumulation in black communities. Studies show that visceral adipose tissue contributes less than 6% and 15% of whole-body venous fatty acid flux in lean and obese subjects, respectively (401-403). The relatively small percentage contribution to fatty acid flux may suggest the latter is occurring (lower rates of total lipolysis protect black men from high VAT accumulation). Another study has also concluded that there may be a resistance to storing VAT in black populations (256). It could be postulated that the lower lipolysis may be an intrinsic protective mechanism in black communities as it has been repeatedly reported that they have lower rates of fat oxidation (303, 324, 404-406). Thus,

having lower rates of lipolysis reduces the circulating fatty acid pool which may prevent the low fatty acid oxidation rate from becoming pathogenic.

The data from this study shows lower rates of lipolysis, plasma triglycerides and visceral adiposity in black men compared to white men. Adjusting for differences in visceral adipose tissue removed ethnic differences in lipolysis. This finding could suggest that the rate of lipolysis and therefore triglycerides, may be lower in black men as a result of less lipolytic VAT storage.

In conclusion, the antilipolytic action of insulin appears to be equal in black and white men in response to a hyperinsulinaemic-euglycaemic clamp across all glucose tolerance groups. Resistance to insulin-mediated lipolysis is therefore not likely to explain the increased risk of T2D in black compared to men. Black men had lower levels of absolute lipolysis; however, when accounting for differences in basal activity using the percentage change assessment, insulin appears to suppress lipolysis at a similar rate in both ethnic groups. The absolute lipolysis rates, when assessed in isolation, are lower in black men and could partially be explained by lower visceral adipose tissue.

Chapter 8: An ethnic comparison of the relationship between peripheral and hepatic insulin sensitivity with visceral fat, hepatic fat and adipose tissue insulin sensitivity

Chapter highlights:

- In both ethnic groups there is a relationship between peripheral and hepatic insulin sensitivity with:
    1. VAT
    2. IHL
    3. Adipose tissue insulin sensitivity
- For point 1 and 2, the association occurs at a lower VAT and IHL quantity which imply that for every unit of fat, insulin sensitivity is lower in BAM.
- The link between adipose tissue insulin sensitivity and peripheral/hepatic insulin sensitivity (point 3) may be independent of VAT or IHL accumulation in BAM as there was no association between adipose tissue insulin sensitivity with VAT or IHL.

Data presented in this chapter have been published:

Bello O, Mohandas C, Shojaee-Moradie F, Jackson N, Hakim O, Alberti KGMM, Peacock JL, Umpleby AM, Amiel SA, Goff LM. Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat. Diabetologia. 2019 May;62(5):835-844.

Bello O, Ladwa M, Hakim O, Marathe C, Shojaee-Moradie F, Charles-Edwards G, Peacock JL, Umpleby MA, Amiel S, Goff LM. Differences in the link between insulin sensitivity and ectopic fat in men of Black African and White European ethnicity. Eur J Endocrinol. 2019 Nov;182 (1):91-101

8.1 Introduction

Ectopic fat, defined as the storage of triglycerides in non-adipose tissue, promotes insulin resistance and has been implicated in the development of type 2 diabetes (T2D) (137, 195). The liver is one of the organs prone to ectopic fat storage. Intrahepatic lipids (IHL) are stored exclusively within hepatocytes and have been linked to hepatic insulin resistance (137, 215, 407). Whilst visceral adipose tissue (VAT), by definition, is not an ectopic depot, increased accumulation of VAT occurs alongside ectopic fat accumulation and has also been linked to insulin resistance and the development of T2D (203, 215, 221, 373, 408). The potential mechanisms which link VAT and IHL to whole-body and tissue-specific insulin resistance have been described in detail in chapter 1. In brief, the portal theory has been proposed as a mechanism which links VAT to insulin resistance. It dictates that highly lipolytic VAT releases fatty acids, adipokines and inflammatory cytokines into the portal vein, which drains into the liver, where they increase hepatic insulin resistance, worsen other aspects of hepatic metabolism and encourage hepatic steatosis. The cytokines and fatty acids may also enter the systemic circulation causing peripheral insulin resistance (182, 409). The portal theory has been supported by molecular (213), animal (212) and human clinical studies (215).

In addition to insulin's action on peripheral and hepatic tissues, it also acts on white adipose tissue to suppress lipolysis. Lipolysis is defined as the hydrolysis of triglyceride molecules into free fatty acids and glycerol (138). Resistance to the antilipolytic effect of insulin has been implicated early on in the progression towards T2D, prior to the development of hyperglycaemia (151-154). Adipose tissue insulin resistance increases systemic lipolysis, which increases the availability of circulating fatty acids which have been shown to promote

peripheral and hepatic insulin resistance (107, 153, 154). This mechanism has been supported by a wealth of *in vivo* studies using various methods which show increasing or decreasing fatty acids reduces and increases peripheral and hepatic insulin sensitivity, respectively (72, 135, 152, 197, 238). Whilst this mechanism appears promising, it has been suggested that the link between adipose tissue insulin resistance and peripheral insulin sensitivity is not a response of increased fatty acids, but rather the result of all tissues becoming resistant at the same time (239); hence the mechanism of lipolysis related insulin resistance have yet to be concluded. Adipose tissue insulin resistance, along with excess caloric intake (high in fats and carbohydrates) and other metabolic dysfunctions, has also been suggested to contribute towards the deposition of ectopic fat and VAT; as described in the spillover theory discussed in chapter 1 (134, 135, 137, 188, 220, 240). The deposition of visceral and ectopic fat may also be a mechanistic link between adipose tissue insulin resistance with peripheral and hepatic insulin resistance.

Black populations have consistently been reported to have less ectopic fat compared to white populations: however, they are thought to be more insulin resistant (324). Until now, *in vivo* studies comparing the relationship between ectopic fat and tissue-specific insulin resistance in black and white adults have been restricted to obese women. Peripheral insulin sensitivity has been shown to associate with VAT in white but not black women (323). Hepatic insulin sensitivity has consistently shown a relationship with VAT and IHL in black women (246, 323), although the relationship in white women was absent in one study (323). It is therefore implied that VAT may not have a role in peripheral insulin resistance in black women but that IHL and VAT appear to play a role in hepatic insulin resistance in black women. No such analysis has been completed in men, and it is not clear if the findings in

women can be extrapolated to men. No *in vivo* study, using precise assessments of substrate fluxes, has compared adipose tissue insulin resistance to peripheral or hepatic insulin sensitivity in black and white populations. Using an estimate of adipose tissue insulin resistance (Adipo IR, calculated as the product of basal NEFA and insulin), a significant positive relationship has been shown with IHL in black and white participants (326). Whether this finding is present when using more refined measures of adipose tissue insulin resistance to lipolysis has yet to be determined.

8.2 Aim

This investigation aims to assess the effect of ethnicity on the relationship between peripheral and hepatic insulin sensitivity with 1) ectopic/visceral fat and 2) the antilipolytic effect of insulin (adipose tissue insulin sensitivity). It also aims to explore the potential mechanisms which lead to insulin resistance by ethnicity. This investigation is to be conducted in black west African (BAM) and white European men (WEM) with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or early type 2 diabetes (T2D).

It could be hypothesised that the association between peripheral or hepatic insulin sensitivity with VAT, IHL and the antilipolytic action of insulin, is weaker in BAM compared to WEM.

8.3 Methods

A full description of the study methods is detailed in chapter 2, which includes the participant inclusion criteria, metabolic assessments and the calculations to derive measures of insulin sensitivity. To summarise, black west African (BAM) and white European men (WEM) were recruited and categorised as normal glucose tolerant (NGT) or impaired glucose tolerant (IGT) based on an oral glucose tolerance test. Participants defined as having type 2 diabetes (T2D) had been diagnosed by their primary care practitioner within five years preceding the screening visit.

Participants attended a two-step hyperinsulinaemic-euglycaemic clamp with a stable [6,6 $^{2}H_{2}$]-glucose and [$^{2}H_{5}$]-glycerol isotope infusion to assess whole-body and tissue-specific insulin sensitivity. The procedure began with a primed continuous [6,6 $^{2}H_{2}$]-glucose and [$^{2}H_{5}$]-glycerol isotope infusion from -120 minutes and ran until 240 and 120 minutes, respectively. The basal phase was defined from -30 to 0 minutes by which time the isotope infusion reaches an equilibrium enrichment with plasma glucose and glycerol. At time 0 minutes, an insulin infusion was initiated at 10mU/m^{2}BSA/min for 120 minutes, defining the first step (low dose insulin infusion) of the clamp. This was followed by a 40mU/m^{2}BSA/min insulin infusion for 120 minutes which defined the second step (high dose insulin infusion) of the clamp. A variable glucose infusion was used to maintain euglycemia at 5mmol/l from 0 to 240 minutes. The final 30 minutes of each step was defined as the steady-state whereby the glucose infused to maintain euglycemia (5mmol/l) equilibrates to the total glucose disposal from the circulation. The glycerol rate of appearance (Ra) and glucose Ra were assessed from samples drawn during the basal phase and the last 30 minutes of the low dose

insulin infusion. The glucose rate of disappearance (Rd) was assessed from samples drawn during the basal phase and the last 30 minutes of the high dose insulin infusion.

Participants also attended a magnetic resonance imaging scan to quantify visceral adipose tissue (VAT) area from the L4/5 anatomical position. Intrahepatic lipid (IHL) percentage was quantified from 8 circular regions of interest covering the superior and inferior surface of the liver.

8.3.1 Calculations

Basal and stimulated whole-body / total lipolysis (predominantly adipose tissue lipolysis) was quantified using the glycerol Ra expressed per m^2 of body surface area (BSA) during the final 30 minutes of basal and low dose insulin infusion. The percentage suppression of adipose tissue lipolysis from basal to the low dose insulin stimulated state was calculated as the primary assessment of adipose tissue insulin sensitivity to lipolysis, termed 'adipose tissue insulin sensitivity' or also defined as 'insulin's antilipolytic action' throughout this chapter (343).

The basal and stimulated endogenous glucose production was quantified using the glucose Ra expressed per m^2 of body surface area (BSA) during the final 30 minutes of basal and low dose insulin infusion. The percentage suppression of endogenous glucose production from basal to the low dose insulin stimulated state was calculated as the primary assessment of hepatic insulin sensitivity (163, 204, 344).

Peripheral glucose disposal was assessed using the glucose Rd expressed per m^2 of body surface area (BSA) during the basal and high dose insulin infusion. The primary assessment of peripheral insulin sensitivity was the percentage increase in glucose disposal

from basal to the high dose insulin infusion (343). The peripheral insulin sensitivity index (PISI) was also calculated during the steady-state of the high dose insulin infusion. This was computed as the peripheral glucose disposal divided by the peripheral insulin concentration (153).

8.3.2 Statistical analyses

Each variable was assessed for normality by ethnic group using the Shapiro-Wilks test and histograms. Pearson's correlations coefficients were determined between pairs of variables with at least one variable having a parametric distribution. In cases where both variables were non-parametric, correlations were assessed using a Spearman's rank correlation coefficient. When combining all glucose tolerance groups to give the largest sample size, linear multiple regression analyses were conducted to determine ethnicity interactions using 'ethnicity* fat depot' and 'ethnicity*adipose tissue insulin sensitivity' as interaction terms where appropriate. In addition to assessing the ethnicity interaction, the Y-intercept of the associations were compared using a t-test based on the intercepts themselves and their standard errors (SE). The test statistic was: t= (difference in intercepts)/(SE(difference). Statistical significance was defined as $p < 0.05$. Data analyses were performed using SPSS software, version 25 (IBM Analytics, Armonk, NY, USA).

8.4 Results

Clinical characteristics for the participants studied in this investigation are found in chapter 3. In brief, BAM and WEM were similar in BMI (p=0.93) which ranged from a normal to obese BMI status, body fat percentage (p=0.94) and fasting glucose (p=0.50). BAM and WEM with NGT and IGT showed no ethnic difference in their 2-hour oral glucose tolerance test plasma glucose (p=0.67), and there were no ethnic differences in the duration of diabetes in those who had been diagnosed with T2D (p=0.74). VAT and IHL were lower in BAM compared to WEM, and this was consistent in all glucose tolerance groups ($p<0.01$ and $p<0.01$, respectively).

8.4.1 Relationships between peripheral insulin sensitivity and visceral fat

Peripheral insulin sensitivity was inversely associated with VAT in BAM and WEM when expressed as peripheral glucose disposal adjusted for insulin (PISI) or the percentage increase in peripheral glucose disposal in participants from all glucose tolerance groups (Figure 17). There was no significant interaction between ethnicity and VAT when modelled in a linear regression with PISI (p=0.22, figure 17A) or peripheral insulin sensitivity assessed as the percentage increase in glucose disposal (p=0.21, figure 17B). An ethnic comparison of the y-intercept of the linear regression between VAT and PISI showed no significant difference with a z score and p value of 1.68 and 0.09 respectively (figure 17A). An ethnic comparison of the y-intercept of the linear regression between VAT and peripheral insulin sensitivity (percentage change in glucose disposal) showed no significant difference with a z score and p value of 1.63 and 0.10 respectively (figure 18B).

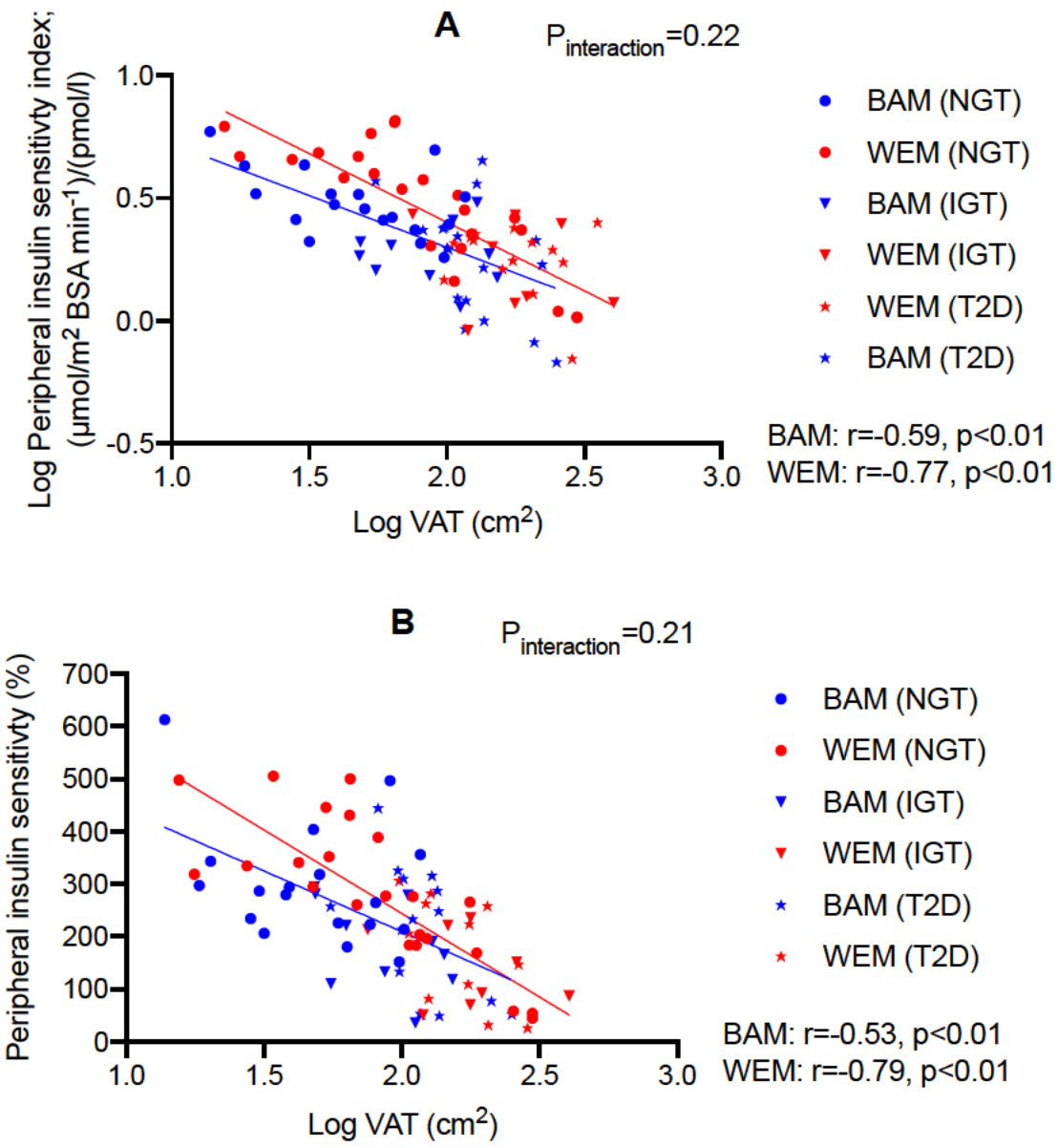


Figure 17: *The relationship between peripheral insulin sensitivity with visceral adipose tissue (VAT).*

Data presented as Pearson's correlation coefficients assessed with log-transformed visceral adipose tissue (VAT)(cm) and peripheral insulin sensitivity index (PISI). The peripheral insulin sensitivity index was assessed during the high dose insulin infusion, and peripheral insulin sensitivity was assessed as the percentage increase in peripheral glucose disposal from baseline to the high dose insulin infusion.

8.4.2 Relationships between peripheral insulin sensitivity and hepatic fat

Peripheral insulin sensitivity was inversely associated with IHL in BAM and WEM when expressed as peripheral glucose disposal adjusted for insulin (PISI) or the percentage increase in peripheral glucose disposal in participants from all glucose tolerance groups (Figure 18). However, the association between IHL and the percentage increase in glucose disposal showed a trend towards significance in BAM ($p=0.06$, figure 18B) but was significant in WEM ($p<0.06$, figure 18B). There was no significant interaction between ethnicity and IHL when modelled in a linear regression with PISI ($p=0.11$, figure 18A) or peripheral insulin sensitivity assessed as the percentage increase in glucose disposal ($p=0.99$, figure 18B). An ethnic comparison of the y-intercept of the linear regression between IHL and PISI showed a significant difference with a z score and p value of 2.09 and 0.04 respectively (figure 18A). An ethnic comparison of the y-intercept of the linear regression between IHL and peripheral insulin sensitivity (percentage change in glucose disposal) showed no significant difference with a z score and p value of 1.29 and 0.20 respectively (figure 18B).

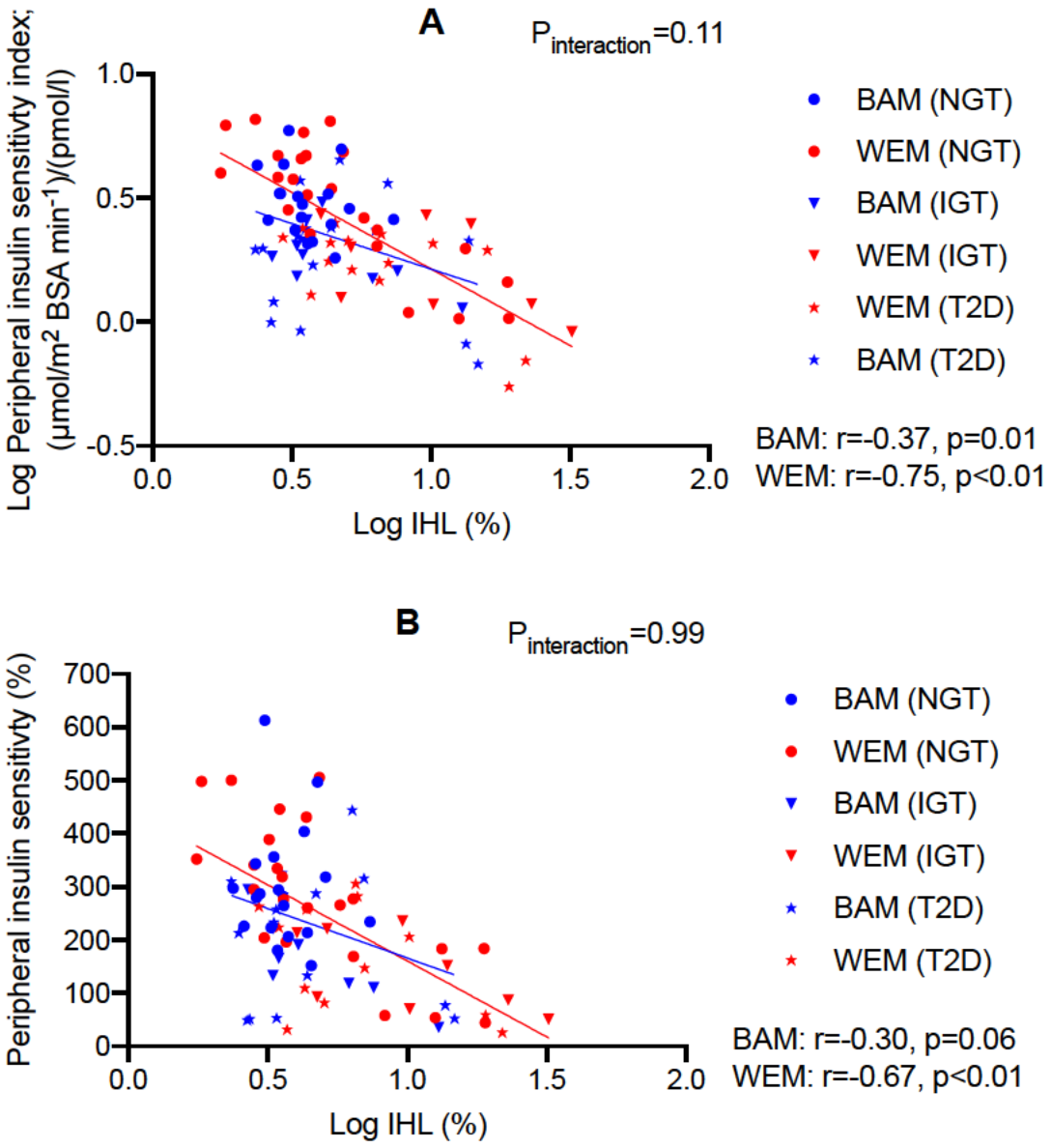


Figure 18: *The relationship between peripheral insulin sensitivity with intrahepatic lipids (IHL).*

Data presented as Pearson's correlation coefficients assessed with log-transformed intrahepatic lipids (IHL)(%) and peripheral insulin sensitivity index (PISI). The peripheral insulin sensitivity index was assessed during the high dose insulin infusion, and peripheral insulin sensitivity was assessed as the percentage increase in peripheral glucose disposal from baseline to the high dose insulin infusion.

8.4.3 Relationships between hepatic insulin sensitivity and fat

The percentage suppression of endogenous glucose production in response to the insulin infusion was the primary assessment of hepatic insulin sensitivity. When correlated with VAT and IHL, there was a significant inverse association to hepatic insulin sensitivity for both ethnicities in participants from all glucose tolerance groups (figure 19A&B). When modelled in a linear regression with hepatic insulin sensitivity, there was no significant interaction between ethnicity and VAT ($p=0.60$) or IHL ($p=0.60$). An ethnic comparison of the y-intercept of the linear regression between VAT and hepatic insulin sensitivity showed no significant difference with a z score and p value of 0.36 and 0.72 respectively (figure 19A). An ethnic comparison of the y-intercept of the linear regression between IHL and hepatic insulin sensitivity showed no significant difference with a z score and p value of 0.09 and 0.93 respectively (figure 19B).

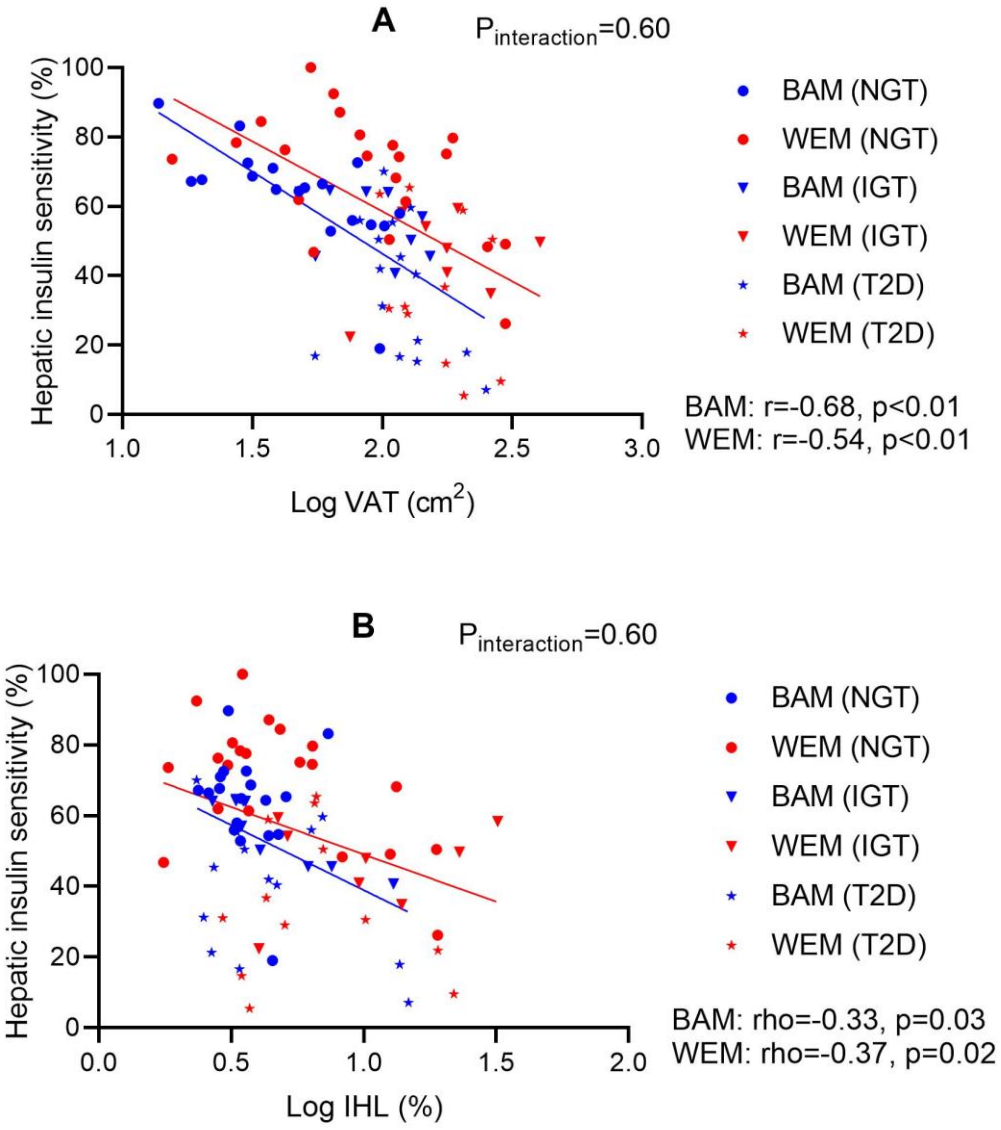


Figure 19: *The relationship between hepatic insulin sensitivity with visceral adipose tissue (VAT) and intrahepatic lipids (IHL).*

Data presented as Pearson's correlation coefficients (r) and spearman's rank coefficient (rho) with log-transformed VAT and IHL. Hepatic insulin sensitivity was assessed as the suppression of endogenous glucose production measured from basal to the low dose insulin infusion.

8.4.4 Relationships adipose tissue insulin sensitivity with peripheral or hepatic insulin sensitivity

The percentage suppression of lipolysis was used as the primary assessment of adipose tissue insulin sensitivity (insulin's antilipolytic action). This showed a significant positive correlation with peripheral insulin sensitivity (percentage increase in peripheral glucose disposal) in both ethnic groups (figure 20A). There was also a positive relationship between adipose tissue insulin sensitivity and hepatic insulin sensitivity (percentage suppression of endogenous glucose production) in BAM and WEM, although this did not achieve statistical significance in the BAM (figure 20B). There was a significant interaction between ethnicity and adipose tissue insulin sensitivity when modelled with peripheral insulin sensitivity ($p=0.01$) but no significant interaction when modelled with hepatic insulin sensitivity ($p=0.15$). An ethnic comparison of the y-intercept of the linear regression between adipose and peripheral insulin sensitivity showed a significant difference with a z score and p value of 3.03 and <0.01 respectively (figure 20A). An ethnic comparison of the y-intercept of the linear regression between adipose and hepatic insulin sensitivity showed no significant difference with a z score and p value of 1.50 and 0.13 respectively (figure 20B).

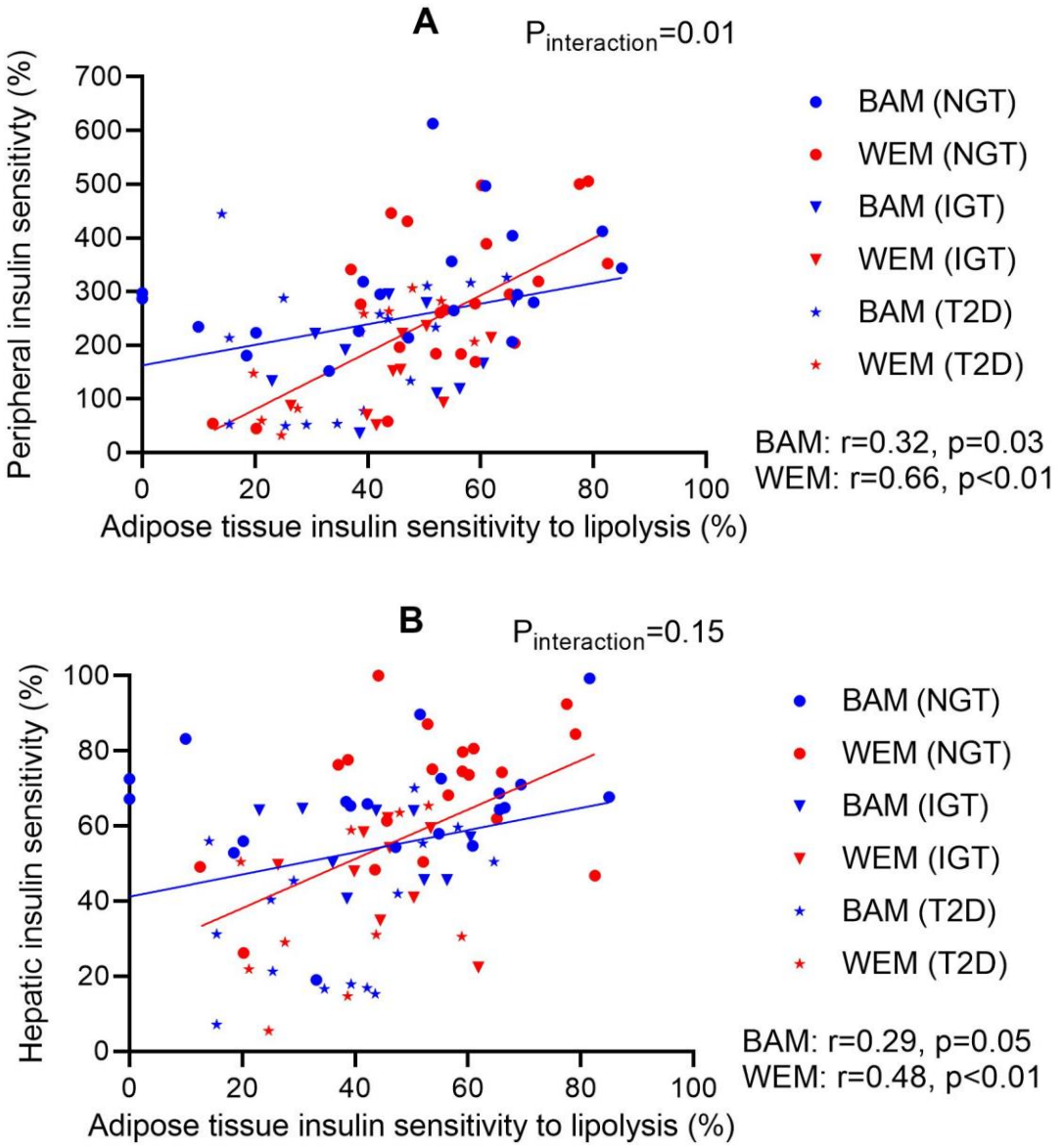


Figure 20: *The relationship between adipose tissue insulin sensitivity with peripheral and hepatic insulin sensitivity.*

Data presented using the Pearson correlation coefficients (r). Peripheral insulin sensitivity was measured as the percentage increase in glucose disposal from basal to the high dose insulin infusion. The suppression of endogenous glucose production and lipolysis were measured from basal to the low dose insulin infusion, which represents hepatic and adipose tissue insulin sensitivity, respectively.

8.4.5 Relationships between adipose tissue insulin sensitivity and ectopic fat

Adipose tissue insulin sensitivity (insulin's antilipolytic action) showed a significant inverse relationship between VAT and IHL in WEM, but there was no significant association in BAM (figure 21A&B). When modelled with adipose tissue insulin sensitivity, there was a significant interaction between ethnicity and VAT ($p=0.03$); however, there was no significant ethnicity and IHL interaction ($p=0.28$). An ethnic comparison of the y-intercept of the linear regression between adipose insulin sensitivity and VAT showed a significant difference with a z score and p value of 4.61 and <0.01 respectively (figure 21A). An ethnic comparison of the y-intercept of the linear regression between adipose insulin sensitivity and IHL showed a significant difference with a z score and p value of 3.65 and <0.01 respectively (figure 21B).

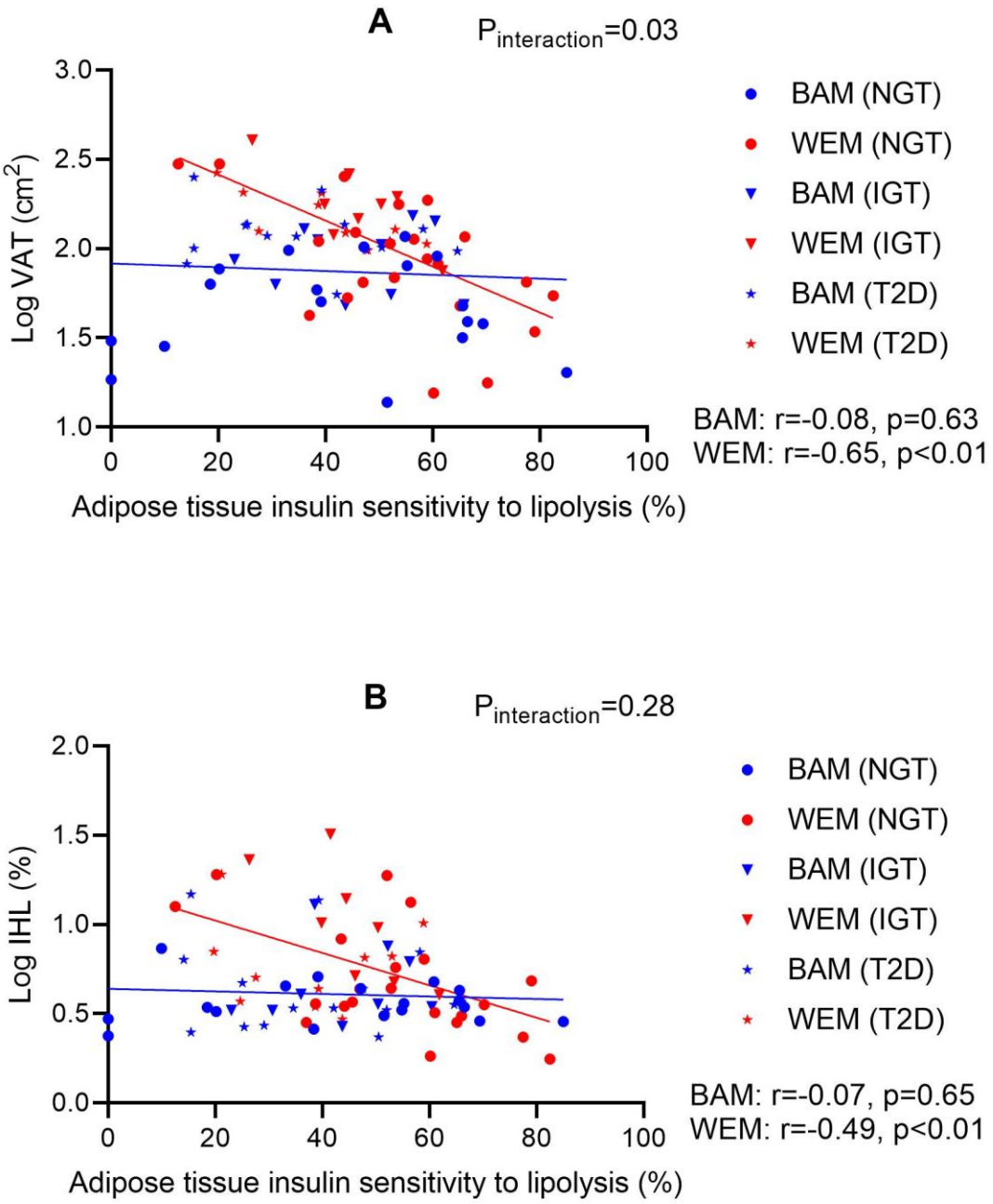


Figure 21: *The relationship between adipose tissue insulin sensitivity with visceral adipose tissue (VAT) and intrahepatic lipid (IHL).*

Data presented as Pearson's correlation coefficients assessed with log-transformed intrahepatic lipid (IHL)(%) and visceral adipose tissue (VAT)(cm).

8.5 Discussion

The analyses in this chapter explore some of the potential mechanisms behind peripheral and hepatic insulin resistance observed in the progression to type 2 diabetes (T2D). Visceral adipose tissue (VAT), hepatic fat (IHL) and adipose tissue insulin resistance have been identified as factors which affect peripheral and hepatic insulin sensitivity and contribute to the development of T2D. Whether these have a causal effect on peripheral and hepatic insulin resistance and evidence for the mechanisms by which this may occur are not conclusive. The present analysis compared the effect of ethnicity on the associations between these variables in black west African and white European men. Prior to this analysis, no such assessment had been conducted in men of black ethnicity using rigorous assessments of insulin sensitivity and fat deposition.

Central adiposity has been linked with insulin resistance and the development of T2D for many years. The accumulation of visceral adiposity has been marked as a key perpetrator in this association (200). Whilst black populations have a higher risk of T2D; they are consistently reported to present with lower VAT (256), as was found in this cohort of participants. The analysis in this chapter showed a significant relationship between peripheral insulin sensitivity and VAT in both ethnic groups. Participants with high VAT had lower peripheral insulin sensitivity, and those with low VAT had higher peripheral insulin sensitivity. This association supports the current thought which implicates high VAT to play a role in the pathophysiology of T2D (203). The lack of an interaction effect suggested that the significant relationship was not ethnically distinct. Therefore, the association between peripheral insulin sensitivity and VAT in black and white men were similar. In contrast, *Goedecke et al.* found no association between peripheral insulin sensitivity and VAT in black

women (323). The difference in findings for black women compared to men may reflect sex differences. In support of sex affecting the associations, a study assessing participants from multiple ethnic groups has shown significant associations between insulin sensitivity and multiple measures of total and regional adiposity in men which are absent in women (410). Another larger study in white adults found that for each incremental increase in a body fat measure, the decline in insulin sensitivity was less in women compared to men (411) which may also imply weaker associations in women. No study has assessed regional adiposity and peripheral insulin sensitivity in black men and women; however, the findings from the reported studies and this analysis imply that sex differences are present in the relationship between regional body fat and insulin sensitivity. This could explain why a significant relationship was found in this analysis but not in the obese black women studied by *Goedecke et al.* (323). The different findings may also be explained by the degree of obesity of the participants. This analysis studied men across a range of BMIs whereas *Goedecke et al.* studied obese women. It could be interpreted that in black women, the association between peripheral insulin sensitivity and VAT is lost in severe obesity. In comparison, when assessing a range of BMIs, a relationship is present. Finally, the inclusion of participants with T2D in these analyses may also explain the difference in findings between this study and *Goedecke et al* (323). Participants with T2D had greater quantities of VAT which may have increased the spread of the VAT data in comparison to *Goedecke et al* (323). Overall, the association and interaction findings from this analysis indicate that there is no ethnic difference in the negative association between VAT and peripheral insulin sensitivity in black and white men. This therefore suggests that the potential detrimental impact of VAT on insulin sensitivity to peripheral insulin sensitivity, is no different in black and white men. No

other study has compared the relationship between VAT and peripheral insulin sensitivity in black and white men.

In addition to VAT, it has been shown that higher levels of hepatic fat are associated with lower peripheral insulin sensitivity in a small group of normal glucose tolerant participants (412) and a study of over 1400 European participants assessed using hyperinsulinaemic-euglycaemic clamps and a fatty liver index (223). In the present study, peripheral insulin sensitivity showed a significant relationship and a trend towards significance; depending on the measure of peripheral insulin sensitivity; with intrahepatic lipids (IHL) which was not ethnically distinct as evidenced by the interaction analysis. The finding of a significant relationship in this study does not agree with Goedecke *et al.* who reported no relationship between liver fat and peripheral insulin sensitivity in obese black and white women (323). The difference in findings may reflect and effect of sex and obesity. Sex and obesity may determine whether an association is present thus the data here contribute to the literature by suggesting a relationship is present in men.

Similar to peripheral insulin sensitivity, hepatic insulin sensitivity has also been reported to associate with VAT, such that individuals with high VAT have lower hepatic insulin sensitivity or individuals with low VAT have higher hepatic insulin sensitivity (203, 215). In the present analysis, the relationship between hepatic insulin sensitivity and VAT was significant in both ethnic groups. Therefore, both ethnic groups showed an association between hepatic insulin sensitivity and VAT with no ethnic difference in this relationship. Black women are consistently reported to display a significant inverse association between hepatic insulin sensitivity and VAT (246, 323). The analyses in this chapter agree with and extend this finding to black men of a variety of glucose tolerances. In addition to the

association with VAT, hepatic insulin sensitivity has also been associated with the storage of triglycerides in the liver, termed intrahepatic lipids (IHL) (128). Whilst populations of black ethnicity are at high risk of developing T2D; they are almost consistently shown to present with lower hepatic fat which is a paradox due to the link between hepatic fat and insulin resistance (256). The analysis in this chapter shows a significant inverse relationship between hepatic insulin sensitivity and IHL in black and white men. The relationship between IHL and hepatic insulin sensitivity was not ethnically distinct, as shown by the interaction analysis. The significant relationship agrees with studies in obese black women which also show a relationship between hepatic insulin sensitivity and hepatic fat (246, 323) therefore, this study extends the conclusion to black men in various glucose tolerance groups and suggest it is not ethnically different. Overall the significant association between hepatic insulin sensitivity with VAT and IHL in both ethnic groups could suggest that high VAT and IHL are equally determinantal to hepatic insulin sensitivity in white and black men. It could be interpreted that the pathophysiology of hepatic insulin resistance via VAT or IHL is similar in black and white men.

In line with the literature, the black men assessed in this study were found to have lower VAT and IHL compared to white men. The significant associations with peripheral and hepatic insulin sensitivity occurred within a lower and smaller range of VAT and IHL content in black men. To reflect this, the intercept of the regression slope appeared to be slightly different by ethnicity. A formal analysis comparing the intercepts by ethnicity showed some evidence for a trend towards significance and a significant ethnic difference between VAT and IHL when modelled with peripheral insulin sensitivity. However, there was no evidence for a statistical difference in the y-intercept for VAT or IHL when modelled

with hepatic insulin sensitivity. Although the statistical tests were not consistently significant, the graphs provide some suggestion that for a given value of VAT or IHL, hepatic insulin sensitivity; and to some extent peripheral insulin sensitivity, was lower in black compared to white men. It could be interpreted that while there were no ethnic differences in the relationship between VAT or IHL with peripheral and hepatic insulin sensitivity, the relationships occur at lower VAT and IHL level in black compared to white men. This may imply that even though VAT and IHL are lower in black men, increasing fat deposition remains detrimental to peripheral and hepatic insulin resistance as higher levels of fat are associated with greater insulin resistance.

In this chapter, the association analysis was limited in that it was not able to assess the direction of the association between VAT or IHL with peripheral or hepatic insulin sensitivity. The 'portal theory' has been proposed to explain the association between VAT and peripheral or hepatic insulin resistance. It postulates that fatty acids, adipokines and cytokines released from VAT drain into the portal vein and cause hepatic and peripheral insulin resistance (182, 199, 212-214). This implies a causal relationship between VAT accumulation and insulin resistance. However, not all data support a causal effect of VAT on insulin resistance. The association may be a result of decreasing insulin sensitivity causing an increase in visceral fat. The 'portal theory' combined with the 'twin cycle hypothesis' both postulate a causal role of IHL accumulation on hepatic insulin resistance (222, 409). Alternatively, the relationship between IHL and hepatic insulin resistance could be due to hepatic insulin resistance increasing IHL accumulation. Regardless of the interpretation of the association direction, the analyses in this chapter show no evidence for ethnic differences in the relationships.

In addition to liver and visceral fat, a resistance to insulin's antilipolytic action (adipose tissue insulin resistance) has also been associated with peripheral and hepatic insulin resistance to glucose homeostasis with a causal role implied (107, 135, 152-154, 197, 238). Data in this chapter contribute to the literature by confirming that this relationship is also present in black men. The interaction analysis suggested that the relationship was significantly weaker in black compared to white men; the ethnic comparison of the y-intercepts also shows evidence for an ethnic difference. The finding of a significant association in black and white men is in keeping with *Bril et al.*, who used less sensitive *in vivo* methods to estimate adipose tissue insulin resistance (326). Whether the antilipolytic action of insulin plays a causal role in insulin resistance to glucose homeostasis in black and white men cannot be assessed here because associations do not assess the direction of relationships. However, the idea that peripheral or hepatic insulin sensitivity cause adipose tissue insulin resistance is not supported by any existing reports. In addition, resistance to the antilipolytic effect of insulin has been shown to occur prior to hyperglycaemia and insulin resistance to glucose metabolism (154). As a result, a resistance to the antilipolytic action of insulin is more likely to drive peripheral and hepatic insulin resistance than the reverse. The association analysis in this thesis suggests that this drive is significantly weaker in black compared to white men. Therefore, the potential effect of increased adipose tissue resistance affecting skeletal muscle glucose uptake and hepatic glucose suppression is less in black compared to white men. This may suggest that adipose tissue insulin resistance plays less of a role in peripheral or hepatic insulin resistance and therefore, T2D development in black men.

Adipose tissue insulin resistance has been described to encourage insulin resistance to glucose homeostasis by increasing FFA availability (220, 237) and increasing ectopic fat accumulation (135, 137, 240). To assess whether the associations between adipose tissue insulin sensitivity with peripheral or hepatic insulin sensitivity are dependent on fat accumulation, the associations between adipose tissue insulin sensitivity and fat were investigated. In this study, the white men showed a significant association between adipose tissue insulin sensitivity with VAT and IHL, in agreement with the literature (153). Therefore, the association between adipose tissue insulin sensitivity with peripheral or hepatic insulin sensitivity may, in part, depend on the accumulation of VAT or IHL. In comparison, the black men showed no significant association between adipose tissue insulin sensitivity and VAT or IHL; even though adipose tissue insulin sensitivity was associated with peripheral and hepatic insulin sensitivity. In black men, this lack of an association between insulin's antilipolytic action and fat, particularly VAT, was statistically different from white men and is in keeping with data in obese women by *Albu et al.* (304). In addition, the ethnic comparison of the y-intercept between adipose tissue insulin sensitivity, VAT and IHL also shows an ethnic difference. The analyses from this study, therefore, suggest that the relationship between insulin's antilipolytic action and peripheral or hepatic insulin sensitivity occurs independent of VAT and IHL in black men. If adipose tissue insulin resistance is a causal mechanism promoting peripheral and hepatic insulin resistance, this may not occur through VAT or IHL accumulation in black men. Other mechanisms may link insulin's antilipolytic action to peripheral or hepatic insulin resistance in black populations which have yet to be elucidated.

The disassociation between adipose tissue insulin sensitivity and IHL or VAT in black men may also suggest that the "spillover" theory may not play a role in the accumulation of VAT or IHL. The "spillover" theory has been used to describe the accumulation of VAT and ectopic fat (including IHL). The theory postulates that dysfunctions in the subcutaneous adipose tissue, which include but are not limited to insulin resistance, prevent fatty acids from being stored safely. The inability to store fatty acids and the increased lipolysis in response to insulin resistance, increase the release of fatty acids into the circulation. The spill over of fatty acids into the circulation increases the deposition of VAT and IHL (137, 189, 192, 195). In black men, the lack of association between adipose tissue insulin sensitivity and VAT or IHL suggests that excessive lipolysis in the presence of insulin may not increase fat deposition. Black men appear to have protection or resistance to storing VAT or IHL available from excess lipolysis. Whilst data in this chapter support this concept, the presence of VAT and IHL, albeit at a lower level, still showed an association with peripheral and hepatic insulin sensitivity which was not ethnically different. The conclusions drawn from the lipolysis data are limited in that it was not localised visceral or subcutaneous fat depot lipolysis. Tracer studies reveal that between 86 to 94% of newly hydrolysed fatty acids into the venous circulation are primarily derived from subcutaneous adipose tissue (401, 403, 413). The high percentage contribution from subcutaneous adipose tissue increases the likelihood that dysfunctional subcutaneous fat was being measured and the potential ethnic difference in the accumulation of visceral and hepatic fat is present.

In conclusion, black men may be protected from accumulating visceral or hepatic fat in response to adipose tissue insulin resistance, as described in the "spillover" theory. Despite the observation that black men present with lower visceral and hepatic fat, the associations

with peripheral and hepatic insulin sensitivity are similar in black and white men. This disagrees with the hypothesis of ethnic differences in the associations and suggests that if visceral and hepatic fat have a causal role in insulin resistance, both ethnic groups are equally susceptible to greater fat deposition causing insulin resistance to glucose homeostasis. In both ethnic groups, adipose tissue insulin sensitivity is also associated with peripheral and hepatic sensitivity; however, in black men, this relationship is weaker and appears to be independent of visceral or hepatic fat. Thus, the potential influence of excessive lipolysis on peripheral or hepatic insulin sensitivity may not be dependent on visceral or hepatic fat accumulation in black men. Diabetes prevention and remission strategies to reduce visceral or hepatic fat may produce ethnically distinct findings in terms of insulin sensitivity to glucose homeostasis.

Chapter 9: Discussion and conclusions

9.1 Discussion

In countries across the globe, black communities are consistently reported to have an increased risk of developing type 2 diabetes (T2D) in comparison to their white counterparts (29, 30, 40, 61, 66). The pathological changes which lead to the development of T2D are complex and have been summarised as the 'ominous octet' (71). An increase in insulin resistance has been shown to occur as part of this process, and this develops at multiple sites. The sites include the peripheral tissues (skeletal muscle and adipose tissue) and the liver, all of which have been the focus of this thesis (71, 76). Populations of black ethnicity have been widely described to display pronounced insulin resistance in comparison to white populations (265), and this is thought to partially explain the increased prevalence of T2D. Studies reporting pronounced insulin resistance in black compared to white participants have been discussed in chapter 1 (section 1.8). The narrative of black communities being more insulin resistant is driven from large studies which assessed insulin sensitivity using surrogate indices such as the homeostatic model assessment (HOMA) which assess insulin sensitivity at the basal state (256, 282-284). It is also based on findings from large studies which have utilised the intravenous glucose tolerance test (IVGTT) with minimal modelling (248, 255, 265, 285, 286, 289, 291-298). These studies include men, women and children as well as individuals across the spectrum of glucose tolerance, a range of BMIs, with a focus on insulin sensitivity of glucose homeostasis. These studies almost consistently report greater insulin resistance to glucose homeostasis in black populations, particularly those which assess healthy participants without T2D. This data has suggested that insulin resistance is an early defect in the progression to T2D, which explain the greater T2D risk (265). The volume of authors who report insulin resistance in black communities has also contributed to the

narrative of pronounced insulin resistance in black populations. These data are a key indicator for an ethnic difference in the physiology of T2D.

In contrast to the narrative of the literature, the findings from this study showed no evidence for an ethnic difference in whole-body or tissue-specific insulin resistance. This data disputes the hypotheses that ethnic differences in insulin resistance are present and may underlie the increased risk of T2D in black communities. Insulin-mediated effects on glucose homeostasis were similar between black and white men in this study. The similar insulin sensitivity was found in each glucose tolerance group, including those without T2D, suggesting that early pronounced insulin resistance is *not* present in black compared to white men. Therefore, this study does not support the suggestion that early impairments of insulin sensitivity alone explain the greater prevalence of T2D in black compared to white men. The findings from this study are derived from a hyperinsulinaemic-euglycaemic clamp with stable isotopic tracers, described as the optimal procedure for assessing whole-body and tissue-specific insulin sensitivity to glucose homeostasis (87, 172). There are multiple expressions of insulin sensitivity which can be calculated based on metabolic size (84, 104, 339) and insulin concentration adjustments (89, 362) and making these adjustments did not alter the findings for no ethnic difference.

The contrast in findings between this study and the literature may reflect the characteristics of the participants studied. One particular observation, also discussed in chapter 4 (section 4.5), is the inconsistency of BMI matching between black and white participants, particularly in the larger studies (256, 282, 284). The average BMI of participants assessed is frequently higher in black compared to white participants. Higher BMI is indicative of greater adiposity (364) and given the association between excess

adiposity and insulin resistance (26, 182), the BMI difference may confound insulin sensitivity findings. The participants assessed in this study were recruited to be similar in BMI by ethnicity, to remove the potential confounding effect. Therefore, these data show that in a BMI matched population, black and white men present with similar insulin sensitivity.

Whilst a lack of BMI matching may partially explain the contrast in findings between the narrative of the literature with this study; there have been several studies which have successfully matched for BMI and still reported greater insulin resistance in their black populations (248, 255, 265, 285, 286, 289, 291-298). The contrast in findings between this study and those who report greater insulin resistance in BMI matched black populations may reflect differences in the methodology used to assess insulin sensitivity. Most of the studies which report greater insulin resistance in black compared to white groups have used indirect methods to assess insulin sensitivity. The indirect methods consist of the surrogate indices and the intravenous glucose tolerance test with minimal model analysis, both of which are based on mathematical modelling of glucose metabolism (87, 168, 172, 173, 414). Pisprasert and colleagues (307) provide comprehensive and convincing evidence which concludes that caution should be taken when using surrogate estimates to assess insulin sensitivity in black populations. In a large cohort of African American and European American participants, they show no ethnic differences in total glucose disposal (whole-body insulin sensitivity) from the hyperinsulinaemic-euglycaemic clamp. However, when assessing insulin sensitivity in the same participants using surrogate indices of insulin sensitivity, the black participants were significantly more insulin resistant. This suggests that surrogate methods overestimate insulin resistance in black participants (307). *Pisprasert et al.* suggest that hyperinsulinaemia,

commonly reported in black populations in response to reduced insulin clearance (266, 366), may alter the mathematical models by which the minimal model and surrogate indices are based on. The pronounced hyperinsulinaemia is observed in the presence of similar glucose in black men which may indicate more insulin is required to produce the same glucose result. It may also indicate a more exponential curve linear relationship between glucose and insulin in comparison to white populations. This suggestion has yet to be assessed in black compared to white communities and is not incorporated into the basis of the minimal model or the surrogate indices. Overall, these data suggest that being of black ethnicity may alter the assumptions used to model insulin sensitivity in indirect assessments. In comparison, the use of the hyperinsulinaemic-euglycaemic clamp in this study and others involves fewer assumptions.

In addition to these ethnic-specific findings which question the use of indirect assessments of insulin sensitivity, the minimal model analysis has been suggested to oversimplify glucose metabolism (172). It assumes a single compartment model for glucose metabolism and is also unable to differentiate between peripheral and hepatic insulin sensitivity (172). Using isotopic tracers with the intravenous tolerance test can create a two-compartment model, and has been suggested to assess peripheral and hepatic insulin sensitivity (172, 415). However, this has been shown to yield unreliable results, potentially due to inadequate suppression of hepatic glucose production and is yet to be validated against the clamp (416, 417). The minimal model outcome from an intravenous glucose tolerance test without isotopic tracers has been validated against the "gold standard" clamp (418); however, the association is significantly weaker in participants with T2D (418) which may be due to low insulin secretion and slow glucose responses. Finally, insulin sensitivity from

this minimal model has also been shown to underestimate insulin sensitivity when compared to the clamp. As a result, it is described as an index or an estimate rather than a direct measurement of insulin sensitivity (87, 172, 418, 419). In conclusion, the impact of black ethnicity on the intravenous glucose tolerance test with minimal model analysis and the limitations of the intravenous glucose tolerance test compared to the clamp, imply that caution should be taken when interpreting findings from an intravenous glucose tolerance test in black participants.

Whilst differences in methodology has been discussed to be a contributing factor to the disagreement between findings from this study and the narrative of the literature, other clamp studies have shown lower whole-body, peripheral and hepatic insulin sensitivity in black compared to white populations (245, 251, 296, 311, 319-322). Therefore, methods alone are not the only reason for the contrasting finings. When comparing the ethnic comparison of insulin sensitivity to the clamp evidence base, there are numerous recurring themes across the results chapters, which appear to affect whether an ethnic difference is present. Sex, BMI status, basal vs stimulated assessments of insulin sensitivity, the different procedures to assess insulin sensitivity and geographical differences, have all been discussed in previous chapters. Whilst the message is not consistent, these factors all appear to play a role in whether ethnic differences in insulin sensitivity and its associations are present. The most consistent recurring theme when comparing the analyses in this thesis to the literature is a potential sex effect. Most of the evidence comparing site-specific insulin sensitivity in black and white populations have been from women. It has almost consistently been reported that ethnic differences in insulin sensitivity are present in women, particularly those who are obese. Women show either no ethnic differences in whole-body, peripheral or hepatic insulin

sensitivity (246, 307, 312, 313, 322, 323), lower whole-body, peripheral and hepatic insulin sensitivity (245, 251, 296, 311, 322) or higher hepatic insulin sensitivity (323) in black compared to white women. In comparison, this study consistently shows no ethnic difference in whole-body or tissue-specific insulin sensitivity in black and white men. This implies that there may be a potential sex and ethnicity interaction affecting whether ethnic differences are present. Ethnic differences in insulin sensitivity, when compared to white participants, may only be present in women. This concept has yet to be captured in a single study comparing tissue-specific insulin sensitivity in individuals of black and white ethnicity; this study is limited to men only, thus the sex ethnicity interaction hypothesis is not conclusive.

In further support of a potential sex ethnicity interaction which determines whether ethnic differences are present; total body adiposity is consistently reported to be higher in women than men (66, 134, 420). However, women are reported to be more insulin sensitive than men. To explain this, detailed studies on body composition show reduced visceral fat and hepatic fat but higher subcutaneous and peripheral adipose tissue in women (195, 420). This is generally regarded as a protective body fat distribution associated with greater insulin sensitivity. Although higher insulin sensitivity is thought to be present in women compared to men; this is not consistent in studies comparing black men and women. A few studies provide data for a tendency towards higher sensitivity in black women compared to men (307, 312, 313) however, studies which directly compare black men and women and found lower insulin sensitivity in women compared to men (353, 421). Overall the sex differences in insulin sensitivity and the contrasting evidence by sex in black communities may imply that participant sex may determine whether ethnic differences are present in black and white participants. The sex interaction is speculative and not fully supported by the literature, which

may be confounded by the other recurring themes listed earlier, such as obesity status and geographical differences. Evidence against a sex*ethnicity interaction is shown in studies which have recruited and analysed whole-body insulin sensitivity in black men and women, of which no ethnic difference in women or men were found (307, 313).

Nevertheless, sex differences may explain differences in the findings between the men in this study and the clamp based literature. Whilst the sex difference is not consistent for each insulin action site, these analyses are the first to produce a consistent message in men such that ethnic differences in whole-body and tissue-specific insulin sensitivity are absent across a range of BMI statuses and glucose tolerances. This is corroborated by numerous studies which show no ethnic difference in whole-body or tissue-specific insulin using clamp methods as discussed in the results chapters. It could, therefore, be concluded that pronounced whole-body or tissue-specific insulin resistance is not likely to contribute towards the greater prevalence of T2D in black men.

Efforts to understand what drives insulin sensitivity to fall as part of the progression to T2D, have implicated visceral fat (VAT) and ectopic fat (particularly hepatic fat (IHL)) (182). Both of these potentially impact on insulin-mediated peripheral glucose disposal and the suppression of endogenous (hepatic) glucose production (186, 197, 203-205). The accumulation of these fat depots has been described to trigger and exacerbate tissue-specific insulin resistance through lipotoxicity and has, therefore, been implicated in T2D pathophysiology and progression (195). Black communities are at high risk of developing T2D; however, they have consistently shown lower VAT and IHL (245-254, 256-258). Lower quantities of fat deposits and the narrative for greater insulin resistance in black

communities is, therefore, a paradox. This may imply that the relationship with insulin sensitivity and VAT or IHL, and potentially the mechanisms which lead to insulin resistance are ethnically distinct. In support of an ethnic distinction relating to adiposity, clinical data show a higher prevalence of T2D in lean black populations and black communities diagnosed as a lower BMI. These data also conflict with views on the development of T2D by suggesting that the quantity of adiposity may not be the sole contributor to T2D development in black communities. Based on mechanistic and clinical findings, it could be hypothesised that the pathophysiology of T2D is less associated with adiposity in black populations and therefore the association between insulin sensitivity and fat deposits would be ethnically distinct (422). To explore this hypothesis, the relationship between insulin sensitivity with VAT and IHL were assessed in black and white men. Despite presenting with lower VAT and IHL in the presence of similar insulin sensitivity, these data showed no ethnic differences in the relationship between peripheral and hepatic insulin sensitivity to glucose homeostasis with VAT and IHL. The potential effect of greater VAT and IHL accumulation on reducing insulin sensitivity did not appear to be ethnically distinct. This may imply that black men may be equally susceptible to insulin resistance and T2D associated with increasing VAT and IHL. This susceptibility may occur at lower levels of VAT and IHL in black men however; greater fat deposition remains associated with lower insulin sensitivity. With an increasing emphasis on treating, and even reversing T2D, by reducing visceral and intra-organ fat (423), the potential for there to be ethnic-specific differences in the magnitude of the response to such interventions needs urgent attention. Although this analysis did not assess the direction of the association between insulin sensitivity and VAT or IHL, the analyses show no ethnic difference in the relationship.

These analyses were also unable to assess the potential mechanisms which may link VAT and IHL to peripheral and hepatic insulin sensitivity. Adipokines and inflammatory markers have been found to be released from VAT (194, 211). Studies comparing black and white participants have shown either no difference or lower adipokines (insulin sensitisers) in black compared to white participants (392, 424-426). Therefore, although the associations with VAT and IHL to insulin sensitivity are similar by ethnicity, the mechanisms may be ethnically distinct.

In this cohort of men, similar whole-body, peripheral and hepatic insulin sensitivity occurred in the presence of lower VAT and IHL in black men. Adjusting for ethnic differences in VAT and IHL resulted in lower insulin sensitivity in black men. This finding was also supported by the association analysis which showed that at any given level of VAT or IHL, insulin sensitivity was lower in black men. These findings and analyses suggest that depositing less VAT and IHL may protect black men from presenting with pronounced whole-body and tissue-specific insulin resistance to glucose homeostasis compared to white men. Further analysis of this potential protective mechanism warrants further investigation and could be used as a target in other high-risk populations.

This is the first report to assess adipose tissue insulin sensitivity to lipolysis in the same cohort of black and white participants in which peripheral and hepatic insulin sensitivity were also assessed. This study, therefore, acknowledges insulin's other, non-glycaemic, metabolic actions which may contribute to T2D development (154). The analysis shows no ethnic difference in adipose tissue insulin sensitivity in any glucose tolerance group between black and white men. Similar to peripheral and hepatic insulin sensitivity, when compared to the literature on adipose tissue insulin sensitivity, the sex of the participants and whether

assessments are made during the basal or insulin stimulated state seemed to determine whether an ethnic difference was present.

In addition to peripheral and hepatic insulin resistance, a resistance to the antilipolytic effect of insulin has been implicated in the development of T2D, shown to occur before hyperglycaemia (154). This adipose tissue insulin resistance is thought to reduce peripheral glucose uptake and the suppression of endogenous glucose production, therefore, peripheral and hepatic insulin resistance, respectively (107, 141, 142, 153, 154, 233). This analysis showed associations between insulin's antilipolytic action and peripheral and hepatic insulin sensitivity in both ethnic groups; however, the association was weaker in black men. This analysis does not assess the direction of the relationship; however, if resistance to insulin's antilipolytic action does promote insulin resistance to glucose homeostasis, then this mechanism may occur to a lesser degree in black men. Resistance to insulin's antilipolytic action may be less involved in the pathogenesis of insulin resistance and therefore, T2D in black men. No study or data suggest that insulin resistance to glucose metabolism causes adipose tissue insulin resistance and adipose tissue insulin resistance has been shown to occur before hyperglycaemia (154). Therefore, it is more likely that excess lipolysis contributes to insulin resistance to glucose metabolism than the reverse. Mechanistic studies suggest adipose tissue insulin resistance is thought to promote peripheral and hepatic insulin resistance by either increasing fatty acid supply or by increasing ectopic fat deposition (135, 137, 240). To assess whether resistance to insulin's antilipolytic action may influence peripheral and hepatic insulin sensitivity through ectopic fat accumulation, an association between adipose tissue insulin sensitivity and fat depots was explored. Significant associations were found in white men which were absent in black men. This suggests that the

potential peripheral and hepatic insulin resistance mediated by excess adipose tissue lipolysis occurs independently of VAT or IHL accumulation in black men. Further studies are required to understand the mechanisms which may link adipose tissue insulin resistance to peripheral and hepatic insulin resistance in black men.

A resistance to insulin's antilipolytic effect in the adipose tissue has been described as part of the dysfunctions in the spillover theory (192). This theory describes the formation of ectopic fat through dysfunctional subcutaneous adipose tissue and highly lipolytic VAT (194, 211). In line with this theory, data from white men showed a significant association between adipose tissue insulin sensitivity with VAT and IHL. In comparison, no association was observed in black men suggesting that the deposition of VAT and IHL may be independent of insulin's antilipolytic action; therefore, the spillover theory may not hold for black men. This analysis also suggests that excess lipolysis in the presence of insulin is not likely to lead to VAT and IHL deposition in black men. Black men may accumulate fat through other mechanisms which require further study. The lack of association combined with the finding of black men having lower VAT and IHL (245-252, 256-258) may suggest that black men have protection from storing fat. And, as discussed earlier, this may further protect black men from having greater insulin resistance to glucose homeostasis. It should be noted that the association analysis could also be interpreted in such a way that high VAT or IHL has a causal effect on adipose tissue insulin resistance in white men. Whilst data on a causal role of IHL is absent; VAT has been described as a highly lipolytic fat depot, thus it could contribute to excess lipolysis in the presence of insulin. However, per unit of mass, VAT contributes a small portion to overall lipolysis; therefore, the direction of the association is likely to be that adipose tissue lipolysis increases VAT and IHL deposition rather than the

opposite. Overall regardless of the direction, the relationship is absent in black men and further assessment into the mechanisms for visceral and intra-organ fat deposition are warranted.

Whilst this study showed no ethnic difference in adipose tissue lipolysis response to insulin from the clamp, absolute rates of lipolysis were lower in black men across all glucose tolerance groups. The lower lipolysis rate could not be explained by insulin; correction for insulin maintained an ethnic difference. VAT has been shown to be more lipolytic than subcutaneous fat; therefore, releasing more fatty acids per unit of mass (211). In line with the literature (245-252, 256-258), the black men assessed in this analysis presented with less VAT. The statistical adjustment of this data for visceral fat removed the ethnic difference, suggesting that the lower absolute rates of lipolysis in black men may be the result of lower VAT. However, as discussed earlier, VATs contribution to total lipolysis is low; thus other mediators of lipolysis such as catecholamines (142) may explain the lower lipolysis in black men.

9.1.1 Strengths and limitations

The finding of no ethnic difference in insulin sensitivity in any glucose tolerance group suggests there are no ethnic differences in the fall of insulin sensitivity during the progression to T2D. However, this study was cross-sectional which limits the interpretation of disease progression that longitudinal data would provide. Even though early insulin resistance in black compared to white men is not supported by the findings in this study due to the similar insulin sensitivity in participants without T2D, the cross-sectional nature of this studies means that it is not clear if participants studied without T2D would go on to develop

T2D and were therefore in the 'early' stages of T2D development. The participants with NGT were not all obese; assessing obese participants could have been used as a marker to suggest that the participants were in the 'early' stages of T2D development. Although participants were allocated as normal glucose tolerant or impaired glucose tolerant, fasting glucose was not accounted for. Therefore, some of the participants with normal glucose tolerance may have also had impaired fasting glucose which is a form of impaired glucose regulation. This may not have been equal across both ethnic groups and constitutes a study limitation.

Another potential limitation of this study could be the lower age in black compared to white men. The contribution of differences in age to the comparison of insulin sensitivity cannot be ruled out completely. As discussed in chapter 3, the trend towards a lower age in black men may be an unavoidable reflection of the epidemiological fact that black populations develop dysglycaemia at a younger age. The ethnic difference in age was found in participants with IGT; however, it is unlikely that age alone removed or created differences between black and white men. Indeed studies have shown that age does not associate with estimates of insulin sensitivity in black or white populations (353). Rather, age-related changes in adiposity are associated with insulin sensitivity (259, 260) which is why participants were recruited to be similar in BMI, and detailed fat depot analyses were conducted.

Outside of VAT and IHL, there are other ectopic fat stores which are associated with insulin resistance and increase as dysfunctional SAT increases, namely intramyocellular lipid (IMCL) (134, 137). This has not been assessed in this thesis and is, therefore, a potential limitation. IMCL was initially thought to increase peripheral insulin resistance (427). Black and white participants have been shown to have similar quantities of IMCL; however, IMCL

does not associate with insulin sensitivity in black cohorts but does in white (317, 428). The role of IMCL in insulin resistance has been questioned due to the finding that the skeletal muscle of athletes, who are insulin sensitive, have high levels of IMCL. Understanding the role, and potentially intracellular mechanisms through biopsy of IMCL may help to understand the associations in black and white ethnicity.

Despite the detailed analysis of region-specific (visceral and hepatic) fat that was assessed in this study and matching participants for BMI, this study lacked a quantification of total lean or fat mass in each participant; matching for BMI does not account for lean mass. As mentioned in chapter 2 and 4, insulin sensitivity is expressed per unit of metabolic mass with a preference towards fat-free mass (FFM). This is because lean mass is a highly sensitive depot in terms of glucose uptake. FFM was not available for all participants and may have limited the interpretations because the insulin sensitivity data could not be adjusted for the insulin sensitive tissues. FFM data was available for participants with NGT and IGT however adjusting for this was not shown to influence ethnic differences in whole-body insulin sensitivity.

The ideal *in vivo* assessment of different adipose tissue depots requires whole body magnetic resonance imaging scanning (429-431). In this study both VAT and SAT were assessed using a single abdominal slice. Although single slice assessments have been validated against whole body measures of regional fat, single slice assessments do increase measurement uncertainty and introduce inaccuracies (430). This is more so apparent for SAT, which unlike VAT, is located in the legs and arms as well as the trunk and constitutes the majority of total fat mass thus, a single slice assessment compromises the ability to detect differences across regions. Accounting for regional subcutaneous adiposity is particularly

important with regards to ethnic comparisons. There is evidence to suggest that black adolescents groups present with greater lower-body SAT in comparison to white counterparts (432) which has also been shown in older adults (433). Therefore, the single slice assessment of SAT is a limitation of this study.

These analyses were also limited by having no assessment for the potential ethnic differences in physical activity. Increasing physical activity is part of the lifestyle changes prescribed for impaired glucose tolerance. Physical activity has independently been shown to improve peripheral insulin sensitivity to glucose homeostasis (236). Whether this plays a role in the effect of ethnicity and insulin sensitivity cannot be assessed in this study. However, a single study has assessed black and white participants following a single bout of physical activity where it was reported that there were no ethnic differences in insulin sensitivity. This may imply that physical activity does not interact with ethnicity to determine insulin sensitivity (318). Another aspect of the participants lifestyle which may have influenced insulin sensitivity but was not assessed was their dietary intake. This study lacked a standardised or rigorous measure of dietary intake before or between the metabolic assessments. A high carbohydrate diet has been shown to increased insulin resistance compared to high fat diets (434). Ethnic differences in dietary intake make have influenced insulin sensitivity and could have been accounted for. A UK based study has shown that proportion of total energy intake derived from carbohydrates and starch was significantly higher in African Caribbean's compared to white Europeans (58). It is therefore plausible that ethnic differences in dietary intake may have influenced the insulin sensitivity findings. The black men assessed here could be consuming a diet which promotes insulin resistance

and that may explain why they have similar insulin resistance despite lower visceral and ectopic fat.

The intracellular aspects of insulin-mediated glucose metabolism by ethnicity were not in the scope of the present study and limit the discussions. Insulin stimulates glucose to undergo both oxidative and non-oxidative disposal (glycogen storage), with the latter shown to fall in T2D. Studies which have assessed non-oxidative glucose disposal during a clamp in black and white participants show no ethnic difference in non-oxidative glucose metabolism (313, 318, 435) particularly when there were no ethnic differences in total glucose disposal. However, in cases where insulin sensitivity was lower in black compared to white participants, non-oxidative glucose disposal was lower in the black participants with no ethnic difference in glucose oxidation (251, 322, 436). Based on this, the present data showing no ethnic difference in insulin sensitivity would be likely to show no ethnic difference in non-oxidative glucose disposal in the black and white men.

One of the strengths of this study and analyses is the use of "gold standard" procedures, namely the two-step hyperinsulinaemic-euglycaemic clamp with stable isotopic tracers. Whilst the procedure has been termed the "gold standard" to assess insulin sensitivity and is highly reproducible, it has been criticised for creating a supraphysiological environment due to the duration and intensity of the hyperinsulinaemia. This disrupts and turns off physiological feedback loops which would be present following a meal. Further studies could confirm the findings from this study by using the triple tracer method during a meal to get an understanding of the ethnicity response to physiological stimuli (437). The findings here indicate that future studies in black and white men are not likely to show an ethnic difference in insulin response. In addition to the supraphysiological environment,

using the procedure to assess hepatic insulin sensitivity has also been criticised. The liver is primarily exposed to insulin from the portal vein, which contains newly secreted insulin from the pancreas, which has yet to undergo first past insulin clearance by the liver. The peripheral insulin infusion during the clamp does not go through the portal vein. It increases the peripheral insulin concentration, which reduces the insulin gradient between the portal vein and peripheral circulation. The insulin detected by the liver does not change much and cannot be accurately quantified without portal vein cannulation. For this reason, caution should be applied when hepatic insulin sensitivity findings are interpreted (128).

Whilst these limitations are apparent, the hyperinsulinaemic-euglycaemic clamp is still regarded as the "gold standard" procedure for the assessment of insulin sensitivity to glucose homeostasis, and its use is therefore a strength of this study (89, 156, 157). By combining the procedure with a glucose and glycerol isotopic tracers, more direct assessments of the changes in glucose or lipid fluxes in response to insulin are determined and used to assess peripheral, hepatic and adipose tissue insulin sensitivity (143, 159). Both the adipose tissue and the liver are sensitive to insulin at much lower concentrations than muscle (94). The insulin concentrations which almost maximally stimulate the muscle (the main component of peripheral tissues responsible for glucose disposal) causes a near maximal suppression of endogenous glucose production and lipolysis, used for hepatic and adipose tissue insulin sensitivity, respectively. Maximal suppression of these actions would not allow for ethnic comparisons to be made; thus, a relatively low and high dose insulin infusion was used in this study. Submaximal or partial suppression of endogenous glucose production and lipolysis could, therefore, be measured and compared between groups as well as near maximal stimulation of glucose disposal. This is the first study which has assessed

peripheral hepatic and adipose tissue insulin sensitivity in a single population of black and white men.

The choice of isotopic tracers used to reflect tissue-specific insulin sensitivity is also a strength in this study. Glucose appearance and disappearance was historically assessed with tritiated glucose; however, there have been reports of inaccuracies from these findings due to the contamination (up to 10%) of non-tritiated glucose. These contaminations underestimate glucose appearance; whereas, [6,6-$^{2}H_{2}$]-glucose is a stable isotopic tracer with less imprecision and is a preferred choice (342, 438). In order to assess lipolysis, either glycerol or fatty acids can be tracers with stable isotopes (143, 144). Glycerol has been identified as a superior tracer for the assessment of lipolysis (rate of appearance). Unlike fatty acids, when glycerol is released from the lipolytic process, it is not readily re-esterified back into triglycerides in adipose tissue as glycerol kinase enzyme is low; therefore, glycerol primarily leaves the cell. In comparison, fatty acids are readily re-esterified back to triglycerides and are likely to underestimate lipolysis if measured (112, 144, 344). Measuring glycerol concentrations alone is also likely to underestimate lipolysis because the plasma glycerol is a reflection of glycerol released from lipolysis and the uptake of glycerol in the liver where it is used as a precursor for gluconeogenesis (161), using the isotope dilution technique prevents this limitation.

The hyperinsulinaemic-euglycaemic clamp assumes that the peripheral insulin infusion is sufficient to suppress any endogenous glucose production. Therefore, the exogenous variable glucose infusion is the only glucose source to the circulation and the variable glucose infusion rate reflects glucose disposal during steady-state conditions. In cases of insulin resistance, residual endogenous glucose production occurs during

hyperinsulinaemia (87, 163, 222) and the glucose infusion rate may underestimate total glucose disposal (M value) as discussed in chapter 4. The use of isotopic tracers allows for the measurement of the residual endogenous glucose production during the high dose insulin infusion. This study found incomplete suppression of endogenous glucose production in some participants; therefore, whole-body insulin sensitivity (the M value) is likely to underestimate insulin sensitivity in these cases which highlight the importance of direct measures of glucose flux. In this analysis, the residual glucose production during the high dose insulin infusion was not ethnically distinct. However, without accounting for it, the true effect of residual glucose production when assessing different ethnicities would have been unknown.

In addition to using a "gold standard" method to measure insulin sensitivity, the assessment of VAT and IHL is also a strength of this study. Magnetic resonance imaging is the optimal non-invasive method for assessing adipose tissue quantity. This method can assess specific sites and differentiate between bone and other tissue types although it does have a higher participant burden in comparison to other imaging methods (e.g. DEXA or CT scans).

Finally, the participant characteristics and the main effect of glucose tolerance on insulin sensitivity provide evidence that the participants assessed in this study were representative of the population. Lower plasma triglycerides and a cardioprotective lipid profile are consistently reported when comparing populations of black ethnicity, as discussed in chapter 3 and 7 (274-277, 293, 358). In this study, the main effects of glucose tolerance on whole-body and tissue-specific insulin sensitivity were consistently significant. Participants with NGT were more sensitive than those with IGT or T2D. Participants with

IGT were either more sensitive or equally resistant than those with T2D which agrees with the literature (72, 439). For both ethnic groups, the spread of insulin sensitivity data was greatest in NGT which also agrees with the literature (440, 441). Overall, these data suggest that the sample was representative of the population which strengthens the credibility of the finding for no ethnic difference in whole-body or tissue-specific insulin sensitivity.

9.1.2 Conclusions

The vast majority of the literature suggests that pronounced insulin resistance is present in black populations in comparison to their white counterparts; in contrast, this study provides evidence against such. This study found no ethnic difference in multiple measures of whole-body or tissue-specific insulin sensitivity between black west African or white European men. This occurred in normal glucose tolerance, impaired glucose tolerance and type 2 diabetes suggesting that pronounced early insulin resistance in black men is not present and may not contribute significantly to the increased prevalence of T2D in this ethnic group.

Insulin sensitivity is thought to decrease with increasing visceral and hepatic fat deposition. The finding of similar insulin sensitivity in black and white men occurred in the presence of lower visceral and hepatic fat which is characteristic of black communities. Adjusting insulin sensitivity findings for the ethnic differences in visceral and hepatic fat generally resulted in lower insulin sensitivity in black west African men. This may imply that lower fat deposition protects black men from presenting with pronounced insulin resistance. The protection theory is supported by the lipolysis data, which shows no relationship between resistance to insulin's antilipolytic action, a potential cause of visceral and ectopic fat deposition, and fat deposition in black men. Ectopic and visceral fat may be stored through mechanisms outside of adipose tissue lipolysis which have not been assessed here. However,

the presence of visceral and hepatic fat is similarly associated to peripheral and hepatic insulin sensitivity, albeit at a lower level in black men, suggesting both ethnic groups have the same potential risk of increasing insulin resistance as visceral and hepatic fat increase and therefore similar pathogenesis of T2D.

In addition to visceral and hepatic fat, a resistance to insulin's antilipolytic action has been associated with insulin resistance to glucose homeostasis. These data show that the association between the antilipolytic action of insulin and insulin sensitivity to glucose homeostasis does appear to be weaker in black men and may imply that this is less of a factor in disease pathogenesis. The mechanisms for this relationship appear to be independent of visceral or hepatic fat accumulation in black but not white men. Further investigation into other mechanisms which may be associated with insulin resistance and a potential target in black men are required.

Overall the data in this thesis suggest fat deposition and lipid metabolism may have a causal role in insulin resistance in black men; however, this does not appear to explain the pronounced prevalence of T2D in black men. Further studies which assess other aspects of the T2D pathophysiology, such as beta-cell function, are warranted to provide targets which reduce the healthcare burden of T2D.

9.1.2.1 Future studies

Although insulin sensitivity to glucose homeostasis was no different between the black and white men studied here, the black men did present with lower quantities of ectopic and visceral fat. Future studies which aim to assess the paradox between lower quantities of fat, but similar insulin sensitivity in black compared to white men, could focus

on assessing the function of adipose tissue per unit of fat, as opposed to the quantity. In line with this suggestion, this thesis does show lower adipose tissue lipolysis in black compared to white men however, this study was unable to identify if this was a result of differences in adipose tissue function or mass. In addition to lipolysis, adipose tissue has been shown to release cytokines which promote insulin sensitivity including adipokines and leptin. Few studies have assessed this function in black and white participants; those which have provide data for lower circulating adipokines and leptin in African Americans compared to their white counterparts (442, 443). No single study has assessed multiple aspects of adipose tissue biology (including lipolysis, inflammation, adipogenesis and oxidation (324)) and their impact on insulin sensitivity in black and white groups. Understanding this may help to explain the paradox between lower ectopic fat but similar insulin sensitivity.

Future studies which aim to understand why type 2 diabetes (T2D) is more prevalent in black groups could re-visit the beta-cell centric model. This model describes that failure of the beta-cell to secrete sufficient insulin is the final common denominator before hyperglycaemia. Multi-site insulin resistance increases the demand on the beta-cell to produce more insulin and contributes towards failure. The data from this thesis shows no evidence for an ethnic difference in insulin sensitivity to glucose homeostasis. The data are limited in that insulin secretion and sensitivity were not evaluated together however; it could be interpreted that there is no ethnic difference in the demand placed on the beta-cell by insulin resistance. The beta-cell centric model identifies other factors which increase beta-cell demand and encourage beta-cell failure which have not addressed in this thesis. These factors may present differently in black and white groups and explain the disparity in

disease prevalence. The other factors include: 1) the incretin response, 2) gastric emptying, 3) inflammation, 4) the gut microbiome and 5) the alpha-cell defect. There is some evidence for an ethnic difference in the incretin response (444) although this is not consistent with some reports of no difference (445). There is some evidence for similar gastric emptying in black and white participants, however this has been assessed in patients with diabetes suffering from Gastroparesis (446). There is also some evidence for differences in inflammatory markers (447) and very little evidence assessing the gut microbiome and the alpha-cells in black and white groups. Overall, further research on these factors thought to influence beta-cell upregulation and failure are required and future research should address the magnitude of their effect on the beta-cell.

Whilst the factors listed encourage the beta-cell to upregulate secretion, once this does occur, dysfunctional kidney metabolism can exacerbate the rate of beta-cell failure by retaining excess glucose. Further studies could assess whether dysfunctional kidney handling is more prevalent in black compared to white populations. This assessment may explain why the progression to T2D occurs significantly faster in black populations as evidenced by the early diagnosis. It is well documented the populations of black ethnicity suffer disproportionately for kidney failure (448), whether that characteristic contributes towards the acceleration in T2D progression requires further study.

The literature which has directly assessed beta-cell function in black and white populations show inconsistent results (449) which may reflect the methods used, or that the ethnic difference in beta-cell function is less pronounced. None the less, the beta-cell failure is a key factor in progression towards T2D and understanding if there are ethnic differences in the physiology that trigger beta-cell failure should be research priority.

Further studies which aim to assess the pathophysiology of T2D in black and white populations may wish to focus on oral or mixed meal-based methods which can simultaneously assess insulin sensitivity, insulin secretion, gastric emptying, the incretin effect. Using physiological tools, as opposed to supraphysiological intravenous glucose assessments, may also help to understand the if hyperinsulinemia is present in black groups during a meal and how that relates to insulin sensitivity and secretion.

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Appendices (publications)

Appendix 1: Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat

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ARTICLE

Black African men with early type 2 diabetes have similar muscle, liver and adipose tissue insulin sensitivity to white European men despite lower visceral fat

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Abstract

Aims/hypothesis Type 2 diabetes is more prevalent in black African than white European populations although, paradoxically, black African individuals present with lower levels of visceral fat, which has a known association with insulin resistance. Insulin resistance occurs at a tissue-specific level; however, no study has simultaneously compared whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity between black and white men. We hypothesised that, in those with early type 2 diabetes, black (West) African men (BAM) have greater hepatic and adipose tissue insulin sensitivity, compared with white European men (WEM), because of their reduced visceral fat.

Methods Eighteen BAM and 15 WEM with type 2 diabetes underwent a two-stage hyperinsulinaemic–euglycaemic clamp with stable glucose and glycerol isotope tracers to assess tissue-specific insulin sensitivity and a magnetic resonance imaging scan to assess body composition.

Results We found no ethnic differences in whole body, skeletal muscle, hepatic or adipose tissue insulin sensitivity between BAM and WEM. This finding occurred in the presence of lower visceral fat in BAM (3.72 vs 5.68 kg [mean difference −1.96, 95% CI −3.30, 0.62]; $p = 0.01$). There was an association between skeletal muscle and adipose tissue insulin sensitivity in WEM that was not present in BAM ($r = 0.78$, $p < 0.01$ vs $r = 0.25$ $p = 0.37$).

Conclusions/interpretation Our data suggest that in type 2 diabetes there are no ethnic differences in whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity between black and white men, despite differences in visceral adipose tissue, and that impaired lipolysis may not be contributing to skeletal muscle insulin resistance in men of black African ethnicity.

Keywords Adipose insulin sensitivity · Black African · Ethnicity · Hepatic insulin sensitivity · Insulin sensitivity · Isotope · Lipolysis · Skeletal muscle insulin sensitivity · Tracer · Type 2 diabetes · Visceral fat

Abbreviations

BSA	Body surface area
BAM	Black (West) African men
MRI	Magnetic resonance imaging
R_a	Rate of appearance
R_d	Rate of disappearance
SAT	Subcutaneous adipose tissue
TTR	Tracer-to-tracee ratio
VAT	Visceral adipose tissue
WEM	White European men

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Introduction

Populations of black African ancestry are disproportionately affected by type 2 diabetes compared with white Europeans [1]. The pathophysiological processes of type 2 diabetes are well documented and include beta cell dysfunction, ectopic fat deposition and insulin resistance of the liver, skeletal muscle

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Research in context

What is already known about this subject?

- Populations of black African ancestry are at higher risk of developing type 2 diabetes compared with their white European counterparts, despite displaying lower visceral and hepatic fat and a favourable blood lipid profile
- The pathophysiology of type 2 diabetes involves insulin resistance that occurs at multiple sites, including skeletal muscle, liver and adipose tissue

What is the key question?

- Do black West African men with early type 2 diabetes present with greater hepatic and adipose tissue insulin sensitivity?

What are the new findings?

- During early type 2 diabetes, there were no ethnic differences in skeletal muscle, hepatic and adipose tissue insulin sensitivity despite greater skeletal muscle mass and reduced visceral fat in black African men
- There appears to be an independent relationship between skeletal muscle insulin resistance and adipose tissue resistance to lipolysis in black African men

How might this impact on clinical practice in the foreseeable future?

- Prevention and treatment strategies that target adipose tissue function may not produce the same impact in black African and white European populations

and adipose tissue [2, 3]; the use of stable isotopes has enabled measurement of these tissue-specific sites of insulin resistance [4]. Black populations typically display lower visceral adipose tissue (VAT) and hepatic fat deposition and a more favourable blood lipid profile [5]. Visceral fat has been positively associated with hepatic and adipose tissue insulin resistance in diabetes and normal glucose tolerance [6]. The lower VAT exhibited in black populations suggests there may be ethnic distinctions in the pathophysiology of type 2 diabetes. There have been several studies comparing tissue-specific insulin resistance in vivo, using the 'gold-standard' hyperinsulinaemic–euglycaemic clamp and stable isotopes, in healthy populations of black and white ethnicities. These have been conducted primarily, although not exclusively, in women and adolescents but have not produced a consistent picture [7–20].

Sex differences in body composition have shown women to express greater central and overall body fat [21]. Accordingly, a sex distinction in the type 2 diabetes phenotype has been shown in black Africans, whereby men display greater insulin sensitivity compared with women [22]. The inconsistences in the findings from adolescent and female populations likely stem from differences in methods and participant body composition. Ethnic comparisons in people with type 2 diabetes are required to inform therapeutic decisions; however, they are also limited to adolescent and female populations [23, 24]. Peripheral insulin-stimulated glucose disposal has been shown to be similar in diabetic adolescents [23] but, to date, no study has compared this in diabetic black and white adults using stable isotope methods. Studies assessing ethnic differences in hepatic insulin sensitivity in type 2 diabetes are few; studies of basal endogenous glucose production have shown no difference in adolescents [23], but no studies have assessed insulin-stimulated suppression of endogenous glucose production and there have been no studies performed in adults with type 2 diabetes. In vivo assessment of adipose tissue insulin sensitivity in type 2 diabetes has been more limited; lower basal NEFA release has been reported in black women [24]; however, no study has assessed insulin-stimulated suppression of NEFA release. To date, no single study has undertaken a comprehensive ethnic comparison of whole body, peripheral, hepatic and adipose tissue sensitivity to insulin using the same study cohort and method.

We aimed to compare tissue-specific sites of insulin sensitivity between black (West) African men (BAM) and white European men (WEM) with early type 2 diabetes using the hyperinsulinaemic–euglycaemic clamp with stable isotopes and to investigate associations between sites of insulin resistance by ethnicity. We hypothesise that in early type 2 diabetes, BAM will have greater hepatic and adipose tissue insulin sensitivity because of their lower VAT deposition compared with their white European counterparts.

Methods

The study was conducted at the Clinical Research Facility, King's College London, London, UK and approved by the London Bridge National Research Ethics Committee (12/LO/1859); all participants provided informed consent. The data were collected as part of the South London Diabetes

and Ethnicity Phenotyping (Soul-Deep) study; recruitment and data collection took place during the period April 2013 to January 2015 [25, 26].

Participants BAM and WEM (self-declared ethnicity, confirmed by grandparental birthplace), aged 18–65 years, BMI 25–35 kg/m^2, with a diagnosis of type 2 diabetes within 5 years, treated with lifestyle advice ± metformin, with HbA_{1c} ≤63.9 mmol/mol (<8%) were recruited from South London primary care practices and selected to be similar in age and BMI. Participants were deemed ineligible if: treated with thiazolidinedione, insulin, chronic oral steroids, beta-blockers; serum creatinine >150 μmol/l; serum alanine transaminase level >2.5-fold above the upper limit of the reference range; positive auto-antibodies for insulin, GAD or A2; sickle cell disease (trait permitted); or were using medications believed to affect the outcome measures. Participants completed a comprehensive medical screening before study entry.

Study design Participants arrived at the Clinical Research Facility in a fasted state, having refrained from eating or drinking anything other than water from 22:00 h the night before. Participants were instructed to refrain from strenuous physical activity in the 48 h preceding the visit, refrain from consuming alcohol in the 24 h preceding the visit and to consume a standardised diet the day prior (~50% of energy from carbohydrate, evenly spread throughout the day, with no more than 30% of daily carbohydrate consumed in the evening meal). Participants using metformin were instructed to stop taking it for 7 days before the visit.

Hyperinsulinaemic–euglycaemic clamp assessments On arrival, participants were weighed in light clothing and their body surface area (BSA) calculated using the Mosteller formula. A cannula was inserted into an antecubital fossa vein to infuse stable isotopically labelled tracers, 20% (wt/vol) dextrose and insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) bound to albumin. A second cannula was inserted retrogradely into the dorsum of the hand, which was placed in a hand-warming unit, to achieve arterialised venous blood samples. A baseline blood sample determined the participant's fasting plasma glucose; if above 5 mmol/l, a sliding scale insulin infusion was used to lower the circulating glucose to 5 mmol/l. At time point −120 min, a primed (2.0 mg/kg), continuous (0.02 mg kg^{-1} min^{-1}) infusion of [6,6-$^2H_2$]-glucose and a primed (0.12 mg/kg), continuous (0.0067 mg kg^{-1} min^{-1}) infusion of [$^2H_5$]-glycerol (CK Gases, Cambridgeshire, UK) were initiated [27]. Basal state blood samples were taken between −30 and 0 min. After infusion of the tracers for 120 min (basal period), a two-stage hyperinsulinaemic–euglycaemic clamp procedure was started at time point 0 min and continued for 4 h (during which the infusion of [6,6-$^2H_2$]-glucose was continued but the infusion of [$^2H_5$]-glycerol was stopped just prior to beginning the second stage of the insulin infusion). Insulin was infused at a rate of 10 mU m^{-2} BSA min^{-1} (initiated with a priming dose of 30 mU m^{-2} BSA min^{-1} for 3 min and then 20 mU m^{-2} BSA min^{-1} for 4 min) during stage 1 (0–120 min) and at a rate of 40 mU m^{-2} BSA min^{-1} (initiated with a priming dose of 120 mU m^{-2} BSA min^{-1} for 3 min and then 80 mU m^{-2} BSA min^{-1} for 4 min) during stage 2 (120–240 min) [19, 28]. Euglycaemia (5 mmol/l) was maintained by variable infusion of 20% (wt/vol) dextrose, which was enriched with [6,6 $^2H_2$]-glucose (8 mg/g glucose with low-dose insulin and 10 mg/g with high-dose insulin) to ensure a constant glucose tracer-to-tracee ratio (TTR). Plasma glucose readings were taken every 5 min, using an automated glucose analyser, to inform adjustment of the glucose infusion rate. Hepatic and adipose tissue insulin sensitivity were evaluated in the low-dose insulin infusion, whole body and peripheral (primarily skeletal muscle) insulin sensitivity were evaluated in the high-dose insulin infusion. Blood samples were collected before beginning the tracer infusions to determine baseline enrichment of glucose and glycerol. At time points −30, −20, −10 and 0 min baseline blood samples were collected, followed by sampling at 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230 and 240 min for the assessment of plasma glucose and glycerol concentrations and enrichments, insulin and NEFA concentrations.

Magnetic resonance imaging Participants attended the magnetic resonance imaging (MRI) unit of Guy's Hospital, King's College London, for the assessment of subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and skeletal muscle mass. Scanning was performed on a 1.5 T Siemens scanner to acquire magnetic resonance images from the neck to the knee (excluding the arms). Participants lay supine with body coils secured on the scanned body area.

For each participant, the MRI scan produced 320 contiguous axial fat and water images, each 3 mm apart. The Dixon-MRI T1-weighted spin-echo sequence includes an echo time of 4.77 ms for the in-phase images, 2.39 ms for the out-of-phase images and a repetition time of 6.77 ms. Magnetic resonance images were analysed using a semi-automated method carried out by Klarismo (London, UK) to quantify SAT and skeletal muscle mass volumes in all images between the neck and knee region and VAT volume in the whole abdominal cavity.

Analyses of samples Plasma glucose concentration was measured by automated glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Yellow Springs, OH, USA). Serum insulin concentration was measured by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare, Camberley, UK). Plasma NEFA were measured by an enzymatic colorimetric assay (Wako Diagnostics, Richmond, VA, USA) on an automated clinical chemistry analyser (ILab 650, Instrument Laboratories, Holliston, MA, USA). The glucose and glycerol enrichment

(TTR) in plasma were measured by gas chromatography-mass spectrometry on an Agilent GCMS 5975C MSD (Agilent Technologies, Wokingham, UK) using selected ion monitoring. The isotopic enrichment of glucose was determined as the penta-*O*-trimethylsilyl-D-glucose-*O*-methyloxime derivative [29]. The isotopic enrichment of plasma glycerol was determined as the tert-butyl trimethylsilyl (tBDMS) glycerol derivative [30].

Calculations Total glucose disposal rate (*M* value in mg kg^{-1} min^{-1}) was calculated as a measure of whole body insulin sensitivity. This was computed as the mean of the glucose infusion rate, corrected for any change in measured plasma glucose concentration, during the final 30 min of the high-dose insulin infusion [28]. Additionally, *M* was adjusted for mean insulin concentration (*M*/I).

Peripheral glucose utilisation (glucose rate of disappearance [R_d], μmol kg^{-1} min^{-1}), endogenous glucose production (glucose rate of appearance [R_a], μmol kg^{-1} min^{-1}) and whole body lipolysis (glycerol R_a, μmol kg^{-1} min^{-1}) were calculated using Steele's non-steady-state equations modified for stable isotopes assuming a volume distribution of 22% body weight [31]. Calculation of glucose kinetics was modified for inclusion of [6,6-$^2H_2$]-glucose in the dextrose infusion [32]. Before calculation of glucose and glycerol kinetics, enrichment and concentrations were smoothed using optical segments analysis [33].

Peripheral glucose utilisation (glucose R_d) was calculated during the basal state and the final 30 min of the high-dose insulin infusion. We used the percentage increase in glucose R_d from basal to the high-dose insulin infusion as a measure of skeletal muscle insulin sensitivity [34].

Endogenous glucose production (glucose R_a) was calculated by subtracting the exogenous glucose infusion rate from total glucose R_a. Glucose R_a was calculated during the basal state and during the final 30 min of the low-dose insulin infusion. Percentage suppression of glucose R_a from basal to the low-dose insulin infusion was calculated as a measure of hepatic insulin sensitivity [35].

Whole body lipolysis (glycerol R_a) was calculated during the basal state and during the final 30 min of the low-dose insulin infusion. Percentage suppression of glycerol R_a from basal to the low-dose insulin infusion was calculated as a measure of adipose tissue insulin sensitivity [34].

The AUC for plasma glucose, insulin and NEFA concentrations during the clamp were calculated using the trapezium rule.

Statistics All variables were checked for normality using the Shapiro–Wilk test and non-normally distributed variables were transformed (log_{10}) for analysis. Normally distributed data are expressed as mean (SD), log-normal data were back transformed to give geometric mean and 95% CI and data that remained skewed after log transformation are expressed as median (interquartile range [IQR]). Ethnic differences between means were determined using the independent samples Student's *t* test for normally distributed data and Mann–Whitney *U* test for skewed data. Mean difference or the ratio of the geometric mean and 95% CI are presented where appropriate. Associations between insulin sensitivity measures and with VAT were tested using Pearson's correlation coefficient. Multiple regression analyses were conducted to adjust for the effect of body composition (VAT and skeletal muscle mass) on insulin sensitivity measures. Linear regression analysis was used to determine the impact of ethnicity (interaction) on the associations between insulin sensitivity measures. A value of $p < 0.05$ was considered statistically significant. Analyses were performed using SPSS software, version 22 (IBM Analytics, Armonk, NY, USA).

Results

Participant characteristics The clinical characteristics of the participants are shown in Table 1. By design, the groups were not statistically different in age, weight and BMI. Waist circumference, BSA, SAT, number of years following diabetes diagnosis, HbA_{1c} and the proportion of those treated with metformin were not different between ethnic groups. Mean VAT mass was 34.5% lower and mean skeletal muscle mass was 11.9% greater in BAM (Table 1).

Whole body, skeletal muscle and hepatic and adipose tissue insulin sensitivity There were no differences in results between the two groups (presented as BAM vs WEM): basal plasma glucose, 5.89 (0.39) vs 5.71 (0.63) mmol/l, $p = 0.38$; insulin, 45.7 (36.8, 56.7) vs 57.3 (39.5, 83.2) pmol/l, $p = 0.24$; and NEFA, 0.48 (0.17) vs 0.55 (0.18) mmol/l, $p = 0.30$. There were no ethnic differences in plasma glucose ($p = 0.89$), insulin ($p = 0.78$) or NEFA ($p = 0.70$) concentrations during the clamp expressed as AUC (Fig. 1). Total glucose disposal rate (*M*), as a measure of whole body insulin sensitivity, did not differ between the ethnic groups (Table 2); the lack of significance continued after adjustment for mean insulin during the high-dose insulin infusion (*M*/I BAM, 0.030 [0.017]) vs WEM, 0.026 [0.011] mg kg^{-1} min^{-1} pmol l^{-1}, $p = 0.46$).

Peripheral glucose utilisation (glucose R_d) during the high-dose insulin infusion (Table 2) and skeletal muscle insulin sensitivity (% increase in peripheral glucose utilisation) were also similar between ethnic groups (Fig. 2a; BAM, 203.5% [126.2] vs WEM, 166.3% [102.5], mean difference of 37.3%, 95% CI −55.6, 130.1; $p = 0.42$). Basal endogenous glucose production (glucose R_a) was similar between BAM and WEM and there were no ethnic differences in endogenous glucose production during the low-dose insulin infusion (Table 2) or in hepatic insulin sensitivity (% suppression of endogenous glucose production) (Fig. 2b; BAM, −36.4% [19.7] vs WEM, −34.8% [20.7], mean difference of −1.61%, 95% CI −17.7, 14.5; $p = 0.84$).

Table 1 Clinical characteristics of BAM and WEM with type 2 diabetes

Characteristic	BAM	WEM	Sample size BAM/WEM	*p*
Age (years)[a]	54.0 (47.9, 60.2)	59.0 (55.5, 62.5)	18/15	0.51
Weight (kg)	90.9 (9.3)	94.2 (11.6)	18/15	0.38
Height (cm)	175.6 (7.6)	176.8 (5.8)	18/15	0.91
BMI (kg/m^2)	29.5 (2.7)	30.1 (2.7)	18/15	0.55
Waist circumference (cm)	103.6 (8.4)	107.5 (8.8)	18/15	0.20
BSA (m^2)	2.08 (0.14)	2.13 (0.15)	18/15	0.40
VAT mass (kg)	3.72 (1.07)	5.68 (2.43)	17/14	0.01*
SAT mass (kg)	11.8 (3.9)	11.8 (2.6)	16/14	0.98
Skeletal muscle mass (kg)[b]	20.7 (2.5)	18.5 (3.0)	17/14	0.03*
Duration of diabetes (years)[a]	3.0 (2.5, 3.6)	3.0 (2.0, 4.0)	18/15	0.74
HbA_{1c} (mmol/mol)	50.4 (7.5)	48.6 (7.8)	18/15	0.50
Systolic BP (mmHg)	138.4 (13.6)	131.8 (13.9)	18/15	0.18
Diastolic BP (mmHg)	86.9 (5.1)	82.9 (10.1)	18/15	0.19
Total cholesterol (mmol/l)	4.17 (0.68)	4.30 (0.72)	18/15	0.61
LDL-cholesterol (mmol/l)	2.37 (0.53)	2.29 (0.70)	18/15	0.71
HDL-cholesterol (mmol/l)	1.19 (0.38)	1.24 (0.24)	18/15	0.66
Triacylglycerol (mmol/l)[c]	1.20 (0.95, 1.52)	1.58 (1.26, 1.97)	18/15	0.09
Treated with metformin (%)	78[d]	53[e]	18/15	0.16

Data expressed as mean (SD) for normally distributed data unless otherwise shown

[a] Median (interquartile range) for skewed distributed data

[b] Skeletal muscle mass was measured from neck to knee excluding arms

[c] Geometric mean (95% CI) for log-transformed data or as percentage of individuals where required

[d] $n = 14$

[e] $n = 8$

*$p < 0.05$

p values were generated using an independent sample Student's *t* test for normally distributed data and Mann–Whitney *U* test for skewed data to compare BAM and WEM

There was no significant difference in basal whole body lipolysis (glycerol R_a) in BAM compared with WEM ($p = 0.08$; Table 2). There were no ethnic differences in lipolysis during the low-dose insulin infusion or in adipose tissue insulin sensitivity (% suppression of lipolysis) (Fig. 2c; BAM, −37.2% [16.0] vs WEM, −37.5% [13.7], mean difference of 0.32%, 95% CI −12.5, 13.1; $p = 0.96$). After adjustment for VAT and skeletal muscle mass, we found no ethnic differences in whole body insulin sensitivity (mean difference, 0.95 mg kg^{-1} min^{-1}, 95% CI −0.48, 2.37; $p = 0.18$) or skeletal muscle insulin sensitivity (mean difference, 82.3%, 95% CI −23.1, 187.8; $p = 0.12$). Similarly when we adjusted hepatic and adipose tissue insulin sensitivity for VAT we found no ethnic differences, with a mean difference of 9.1%, 95% CI −9.8, 28.0, $p = 0.33$ and 8.4%, 95% CI −5.7, 22.4, $p = 0.23$, respectively. Correlation analysis of hepatic insulin sensitivity with VAT was only significant in BAM (BAM, $r = -0.55$, $p = 0.04$; WEM, $r = -0.23$, $p = 0.50$) and when we correlated VAT with adipose tissue insulin sensitivity we found no association in BAM ($r = -0.13$, $p = 0.66$) or in WEM ($r = -0.60$, $p = 0.09$).

Associations between tissue-specific sites of insulin sensitivity

As shown in Fig. 3a, we found a significant correlation between skeletal muscle (% increase in R_d) and hepatic insulin sensitivity (% suppression of R_a) in both ethnicities. We found no significant correlation between skeletal muscle and adipose tissue insulin sensitivity (% suppression of glycerol R_a) in BAM; however, there was a strong correlation in WEM (Fig. 3b). There were no significant correlations between hepatic and adipose tissue insulin sensitivity in either ethnicity (Fig. 3c). We further explored the impact of ethnicity on these associations using regression analysis and found no significant ethnicity interaction for the impact of hepatic tissue insulin sensitivity on skeletal muscle insulin sensitivity ($p = 0.82$), adipose tissue insulin sensitivity on skeletal muscle insulin sensitivity ($p = 0.26$) or adipose tissue insulin sensitivity on hepatic insulin sensitivity ($p = 0.84$).

Discussion

To our knowledge this is the most comprehensive comparison of whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity in a single study between adults of black African and white European ethnicity with early type 2 diabetes. We have found, in BAM and WEM with early type 2 diabetes and of similar BMI and age, comparable whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity despite lower VAT deposition and greater skeletal muscle

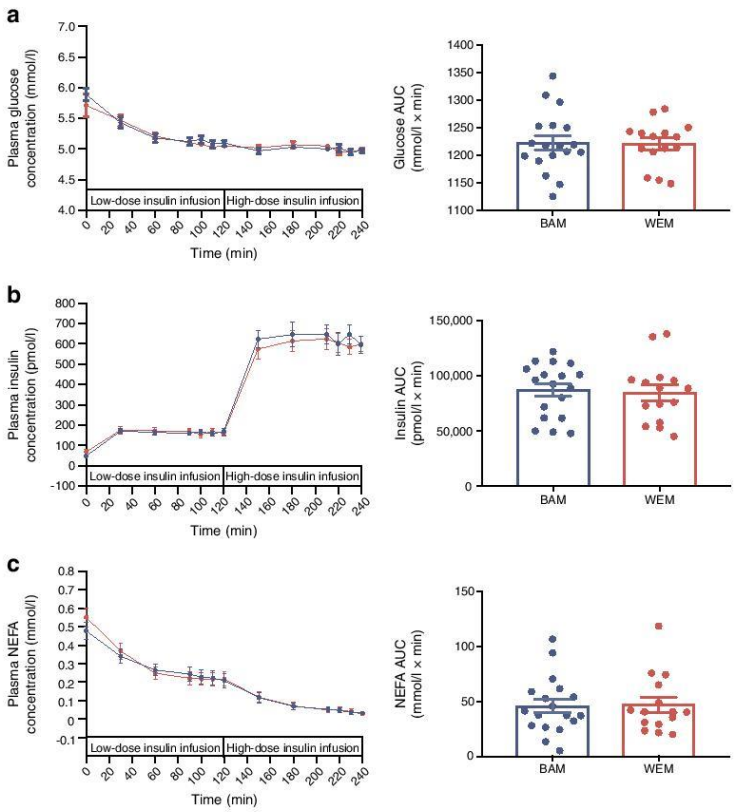

Fig. 1 Plasma glucose (**a**), insulin (**b**) and NEFA (**c**) concentrations for BAM and WEM with type 2 diabetes at baseline, and with low-dose and high-dose insulin infusions during the hyperinsulinaemic–euglycaemic clamp. Data are expressed as mean (SEM) for each time point and glucose, insulin and NEFA AUC. Blue circles, BAM; red circles, WEM. Statistical significance between BAM and WEM was assessed using an independent sample Student's *t* test; there were no significant differences between BAM and WEM

Table 2 Two-stage hyperinsulinaemic–euglycaemic clamp assessment of insulin sensitivity in BAM and WEM with type 2 diabetes

Measurement	Basal				Hyperinsulinaemic–euglycaemic clamp			
	BAM $n = 15$	WEM $n = 12$	Mean difference or ratio of the geometric mean (95% CI) (BAM − WEM) (95% CI)	*p*	BAM $n = 18$	WEM $n = 15$	Mean difference (BAM − WEM) (95% CI)	*p*
Glucose disposal rate (M; mg kg^{-1} min^{-1})	–	–	–	–	4.52 (2.07)	4.00 (1.70)	0.52 (−0.82, 1.89)	0.44
Peripheral glucose utilisation (R_d; μmol kg^{-1} min^{-1})	–	–	–	–	26.8 (10.4)	24.2 (8.5)	2.60 (−4.22, 9.41)	0.44
Endogenous glucose production (R_a; μmol kg^{-1} min^{-1})	8.82 (1.49)	9.25 (1.66)	−0.43 (−1.69, 0.81)	0.48	5.76 (1.73)	6.50 (2.34)	−0.74 (−2.18, 0.71)	0.31
Lipolysis (glycerol R_a; μmol kg^{-1} min^{-1})	1.51 (1.31, 1.75)	1.82 (1.55, 2.15)	0.83 (0.67, 1.02)	0.08	1.06 (0.47)	1.18 (0.33)[a]	−0.12 (−0.43, 0.19)	0.43

Data expressed as mean (SD) for normally distributed data and geometric mean (95% CI) for skewed data

M values and glucose R_d assessments were derived from the high-dose insulin infusion (40 mU m^{-2} BSA min^{-1}), glucose and glycerol R_a assessments were derived from the low-dose insulin infusion (10 mU m^{-2} BSA min^{-1}) of the hyperinsulinaemic–euglycaemic clamp and at baseline

[a] WEM sample size = 13

p values were generated using an independent sample Student's *t* test to compare BAM and WEM

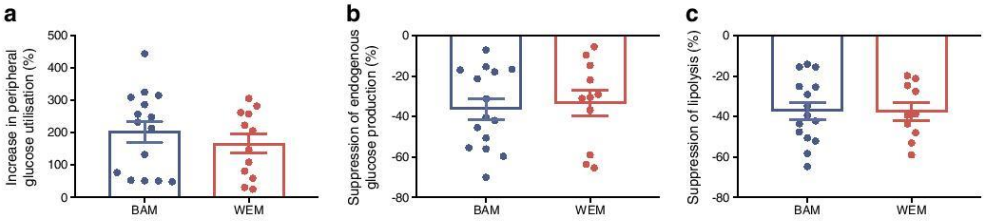

Fig. 2 Insulin-mediated peripheral glucose uptake (**a**), suppression of endogenous glucose production (**b**) and suppression of lipolysis (**c**), calculated as percentage change from basal to the low- or high-dose insulin infusion. Differences in sample size from Table 2 are due to a small number of participants missing basal data owing to the administration of a sliding scale insulin infusion to achieve euglycaemia 5 mmol/l prior to beginning the clamp. Data are expressed as mean (SEM). Statistical significance between BAM and WEM was assessed using an independent sample Student's *t* test; there were no significant differences between BAM and WEM

mass in BAM. In addition, we have shown ethnic differences in the associations between tissue-specific sites of insulin sensitivity, which adds to the concept of ethnic distinctions in type 2 diabetes pathophysiology.

Lower visceral fat deposition has been extensively reported in black populations compared with other ethnic groups [5] and we hypothesised that, consequently, BAM would exhibit greater adipose tissue and hepatic insulin sensitivity. Although our data did not show a significant relationship between adipose insulin sensitivity and VAT, which may be due to the sample size, we did detect lower VAT and a possible, but non-significant, trend towards lower basal lipolysis in our BAM group, which agrees with the majority of the literature. However, we did not find greater adipose tissue insulin sensitivity (even after adjustment for VAT), which contrasts with the findings of a study in black women [14], notably this study used a lower insulin dose than ours and the women were free of type 2 diabetes, which may explain some of the inconsistencies

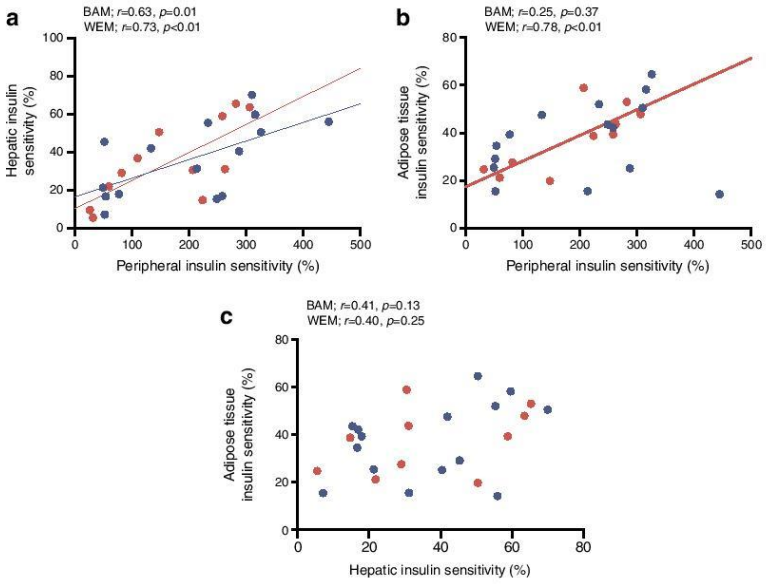

Fig. 3 Associations between tissue-specific insulin sensitivities during the hyperinsulinaemic–euglycaemic clamp in BAM and WEM with early type 2 diabetes: (**a**) peripheral (calculated as the percentage increase in glucose R_d from basal to high-dose insulin infusion, 40 mU m^{-2} BSA min^{-1}) and hepatic insulin sensitivity (calculated as the percentage suppression of glucose R_a from basal to low-dose insulin infusion, 10 mU m^{-2} BSA min^{-1}); (**b**) peripheral and adipose tissue (calculated as the percentage suppression of glycerol R_a from basal to low-dose insulin infusion, 10 mU m^{-2} BSA min^{-1}) insulin sensitivity; and (**c**) hepatic and adipose tissue insulin sensitivity. Data expressed using Pearson's correlation coefficient with corresponding *p* values for BAM and WEM. Sample size: BAM, $n = 15$; WEM, $n = 12$ (except for adipose tissue insulin sensitivity analyses, where $n = 10$ for WEM). Blue dots and regression line, BAM; red dots and regression line, WEM

between our findings. Our men had recently been diagnosed with type 2 diabetes, thus pathophysiological changes may be present that are not seen in non-diabetic groups. Likewise, the presence of diabetes may be important in the lack of greater hepatic insulin sensitivity in our black men, as per our hypothesis. Our findings agree with studies in adolescents [7, 10–13, 20, 23] and a single study in lean non-diabetic women [18] but do not agree with studies in obese non-diabetic women [17, 19] suggesting that, in addition to glycaemic status, body composition may also play a role in the ethnic comparison. We did detect ethnic differences in the association between VAT and hepatic insulin sensitivity however our results were consistent after adjustment for VAT, hence future studies controlling for other ectopic fat depots could help us to understand the impact of adiposity and ethnicity in type 2 diabetes.

Another finding from this study is the lack of ethnic differences in whole body or skeletal muscle insulin sensitivity, which remained even after we adjusted for differences in VAT and skeletal muscle mass. An extensive literature base exists in which black populations are noted to exhibit pronounced insulin resistance compared with other ethnic groups; however, the majority of these studies have used methods which estimate, rather than directly measure, insulin sensitivity [36] and even in studies using the hyperinsulinaemic–euglycaemic clamp method, mixed results are reported [37]. Again, the type 2 diabetes status of our participants is important here, as the presence of diabetes may have attenuated any pre-morbid ethnic differences in insulin sensitivity. This suggestion is supported by the results from a large study of diabetic and non-diabetic populations [38] in which the intravenous glucose tolerance test was used to assess insulin sensitivity. While ethnic differences were present in the non-diabetic state [38], they were absent in type 2 diabetes [39], suggesting that by the end of the glucose tolerance spectrum ethnic differences in insulin sensitivity may have dissipated. It is also reasonable to propose that the adiposity status of our participants may explain the absence of ethnic differences in insulin sensitivity. We aimed to achieve a similar mean body mass index in our two ethnic groups. Their body weights were, on average, in the overweight and obese range, as is typical for people with type 2 diabetes, hence the impact of excess adiposity may also have attenuated any ethnic differences, as discussed in other studies comparing ethnicity in populations with type 2 diabetes [23]. However, insulin sensitivity data, which have been stratified for obese and non-obese in type 2 diabetes, have also shown no ethnic differences [39] suggesting that our result is real and driven more by the presence of type 2 diabetes. In addition to obesity status, we were able to assess skeletal muscle mass which was found to be higher in BAM. Having the same whole body and skeletal muscle insulin sensitivity in the presence of greater skeletal muscle mass, and having the same hepatic and adipose sensitivity in the presence of reduced VAT, suggests that the BAM may be more insulin resistant when adjusted for lean mass [23, 40]; however, we did not find this and there may be other confounding factors, such as muscle and hepatic lipid content, that explain this finding but that are not investigated here.

Increased NEFA release (lipolysis), which occurs during excess adiposity, particularly visceral adiposity, has been shown to impair glucose homeostasis through the process of lipotoxicity. The NEFAs impair insulin signalling and lead to skeletal and hepatic insulin resistance, contributing to the pathophysiology of type 2 diabetes [41–43]. We would therefore expect to see a significant relationship between lipolysis and both skeletal muscle and hepatic insulin sensitivity. Our data show a strong association between lipolysis and skeletal muscle insulin sensitivity in WEM that was not present in BAM. This may suggest an independent relationship of adipose tissue lipolysis with skeletal muscle glucose uptake in BAM and may imply that mechanisms other than lipotoxicity are central to the development of hyperglycaemia in BAM. We do, however, acknowledge that our regression analysis failed to support an impact of ethnicity on the relationship between lipolysis and skeletal muscle sensitivity, which may result from the small sample size in our study. Although we have not directly measured lipotoxicity, as this involves a combination of increased NEFA availability and uptake into the muscle, the concept of an independent relationship between glucose and lipid metabolism is supported by a number of studies that have identified the presence of hyperglycaemia in the absence of pronounced ectopic fat, particularly visceral fat [26]. Further investigation of muscle lipid uptake, insulin signalling and ectopic fat deposition would help to improve our understanding of the impact of lipotoxicity on skeletal muscle insulin resistance in black populations.

The strengths of this study lie in our use of the hyperinsulinaemic–euglycaemic clamp with stable isotope infusions to directly assess and compare tissue-specific insulin sensitivity in vivo in a single study [28]. In particular, the use of a glycerol tracer as opposed to a NEFA tracer allows for a direct measure of NEFA release because, unlike NEFA, glycerol is not recycled back into triacylglycerol [44]. Using a two-stage hyperinsulinaemic–euglycaemic clamp allowed for a low- and high-dose insulin infusion to be applied, enabling quantification of suppression of endogenous glucose production and lipolysis, which is missed when only a high-dose insulin infusion is used [45]. All of the studies assessing hepatic and skeletal insulin sensitivity in adolescents have used a single high-dose insulin clamp in which endogenous glucose production is near maximally suppressed, preventing assessment of suppression of endogenous glucose production. Our study is necessarily small because of the complexity of our protocol; however, it is comparable with other studies using these methods in type 2 diabetes [23, 24]. Furthermore, these data were collected as part of a larger study powered to investigate ethnic differences in beta cell function (reported

elsewhere by Mohandas et al [26]) and we acknowledge that our sample size may hinder the conclusions we can draw from these data. While we made an effort to control the dietary intake prior to the metabolic assessments, which may have impacted on metabolism and insulin sensitivity, we did not undertake a formal analysis of adherence to this aspect of the protocol. We also must consider that the insulin dosage we used in our high-dose stage may not have been sufficient to induce full suppression of endogenous glucose production for our most insulin-resistant participants; however, on average we achieved 80% suppression from the basal level.

In conclusion, we have found that in early type 2 diabetes there are no ethnic differences in insulin sensitivity between BAM and WEM despite BAM having lower visceral fat and higher skeletal muscle mass. While adipose tissue lipolysis is strongly associated with skeletal muscle insulin sensitivity in WEM, there is less evidence for an association in BAM, suggesting an independent relationship between glucose and lipid metabolism may exist within the development of type 2 diabetes in this ethnic group.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Contribution statement LMG, SAA, JLP and AMU formulated the research question and study design. LMG, SAA, JLP, AMU, OB, KGMMA, CM, FS-M, NJ and OH contributed towards the acquisition, analysis, or interpretation of data for the manuscript. All authors contributed to drafting or critically revising the manuscript before giving final approval of the version to be published. All authors agree to be accountable for all aspects of their work and will ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. LMG is the guarantor of this work, had full access to all the data and takes full responsibility for the integrity of the data and the accuracy of data analysis.

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Appendix 2: Differences in the link between insulin sensitivity and ectopic fat in men of Black African and White European ethnicity

Clinical Study | O Bello and others | Differences in the link between insulin sensitivity and ectopic fat | 182:1 | 91–101

Differences in the link between insulin sensitivity and ectopic fat in men of Black African and White European ethnicity

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Abstract

Objectives: In men of black west African (BAM) and white European (WEM) ethnicity, we aimed to (1) compare adipose tissue, peripheral and hepatic insulin sensitivity and (2) investigate associations between ectopic fat and insulin sensitivity by ethnicity.

Design and methods: In overweight BAM (n = 21) and WEM (n = 23) with normal glucose tolerance, we performed a two-step hyperinsulinaemic–euglycaemic clamp with infusion of [6,6 $^2H_2$]-glucose and [$^2H_5$]-glycerol to measure whole body, peripheral, hepatic and adipose tissue insulin sensitivity (lipolysis). Visceral adipose tissue (VAT), intrahepatic lipids (IHL) and intramyocellular (IMCL) lipids were measured using MRI and spectroscopy. Associations between insulin sensitivity and ectopic fat were assessed using Pearson's correlation coefficient by ethnicity and regression analysis.

Results: There were no ethnic differences in whole body or tissue-specific insulin sensitivity (all $P > 0.05$). Suppression of lipolysis was inversely associated with VAT and IHL in WEM but not BAM (VAT: WEM $r = -0.68$, $P < 0.01$; BAM $r = 0.07$, $P = 0.79$. IHL: WEM $r = -0.52$, $P = 0.01$; BAM $r = -0.12$, $P = 0.63$). IMCL was inversely associated with skeletal muscle insulin sensitivity in WEM but not BAM (WEM $r = -0.56$, $P < 0.01$; BAM $r = -0.09$, $P = 0.75$) and IHL was inversely associated with hepatic insulin sensitivity in WEM but not BAM (WEM $r = -0.53$, $P = 0.02$; BAM $r = -0.13$, $P = 0.62$).

Conclusions: Ectopic fat deposition may play a lesser role in reducing insulin sensitivity in men of black African ethnicity and may not be driven by lipolysis. Resistance to storing VAT, IHL and IMCL may enable men of black African ethnicity to maintain comparable insulin sensitivity to white Europeans.

European Journal of Endocrinology (2020) **182**, 91–101

Introduction

The risk of developing type 2 diabetes (T2D) is disproportionately high in populations of black compared to white ethnicity (1, 2). In black populations, T2D is more likely to occur within the normal BMI range (19.5–24.9 kg/m^2) (3) and at a lower waist circumference (4) compared to white groups.

Insulin resistance for carbohydrate metabolism is a well-established early defect in the pathogenesis of T2D (5). Resistance to the antilipolytic effect of insulin in adipose tissue has also been identified as an early defect, occurring prior to the onset of hyperglycaemia (6). Adipose tissue insulin resistance results in increased fatty

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acid release, with deposition in non-adipose tissue sites as ectopic fat (7, 8). This is known to trigger and exacerbate insulin resistance (9). Several theories have been proposed to explain the development of ectopic fat and insulin resistance. The 'spillover theory' proposes that multiple dysfunctions of s.c. adipose tissue (SAT), including insulin resistance (10), allow fatty acids to be deposited as visceral adipose tissue (VAT) (7, 11). Dysfunctional SAT combined with highly lipolytic VAT leads to the release of fatty acids into the portal and peripheral circulations. The 'portal theory' proposes that delivery of fatty acids from VAT to the liver, via the portal circulation, results in the accumulation of intrahepatic lipid (IHL), which subsequently leads to the development of hepatic insulin resistance (12, 13, 14). Fatty acids entering the peripheral circulation are understood to lead to fat deposition within skeletal muscle cells (termed intramyocellular lipids, IMCL) (13, 15). Whilst there is compelling evidence linking IMCL with peripheral insulin resistance (16, 17), there is debate in this field due to observations that athletes, who are highly insulin sensitive, present with relatively high IMCL levels (18).

Compared to populations of white ethnicity, black populations are reported to exhibit lower ectopic fat (namely VAT and IHL) (19, 20, 21, 22, 23), yet large cohort studies indicate that they display pronounced insulin resistance (24), creating a paradox. Studies using more sensitive measures of insulin resistance at a tissue-specific level, alongside measurement of ectopic fat depots related to T2D are scarce in non-diabetic black populations and have been restricted to obese women (25). These have reported no ethnic differences in peripheral insulin sensitivity, but lower hepatic insulin sensitivity in white compared to black women. Furthermore, they report that peripheral insulin sensitivity does not associate with either VAT or IMCL in black women but hepatic sensitivity does associate with VAT and IHL, suggesting that VAT and IHL play a key role in hepatic insulin resistance in black women (25). Similarly, *in vivo* studies assessing adipose tissue insulin sensitivity are mainly confined to obese women. They have provided inconsistent results, showing either no difference (26, 27, 28) or reduced lipolysis (29, 30, 31) in black compared to white populations.

While studies in women provide persuasive evidence of ethnic distinctions in the pathophysiology of T2D, gender differences in physiology (greater hyperinsulinaemia and insulin resistance in women (32, 33)) and body composition (less VAT and more SAT in women (34)) suggest investigations in black men are required. We aimed to assess and compare whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity in normally glucose-tolerant black west African (BAM) and white European men (WEM) and to evaluate relationships between tissue-specific insulin sensitivity with VAT, IHL and IMCL, to explore ethnic distinctions in the pathophysiology of type 2 diabetes.

Subjects and methods

Participants

The participants included in this analysis were recruited as part of the South London Diabetes and Ethnicity Phenotyping (Soul-Deep) study, phase II (35). The aim of the Soul-Deep II study was to investigate ethnic differences in insulin sensitivity, beta-cell function and ectopic fat deposition in men of Black (west) African (BAM) and white European (WEM) ethnicity. The study was approved by the London Bridge National Research Ethics Committee (15/LO/1121). Data collection took place between April 2016 and May 2018. Participants were recruited from local GP practices, newspaper advertisements, King's College London university staff and student email, religious groups, leafleting and posters where permitted. All participants provided informed consent prior to any study procedures.

Non-diabetic Black (west) African (BAM) and white European men (WEM) aged 18–65 years, with a BMI between 20 and 40 kg/m^2 were eligible to take part; the aim of recruitment was to achieve comparable BMI and age between the ethnic groups, without targeting a specific weight status. Ethnicity was defined by self-reported parental and grandparental birthplace; normal glucose tolerance was confirmed by a 2-h plasma glucose <7.8 mmol/L following a 75 g oral glucose tolerance test at screening. Participants were excluded if they were being treated with any medications known to affect the study outcomes, suffering from kidney or liver damage (serum creatinine >150 µmol/L or serum alanine transaminase level >2.5-fold above the upper limit of the reference range), or were unwilling and/or unable to comply with the study protocol.

Prior to each visit, participants were required not to eat after their usual carbohydrate-containing evening meal (no less than 10 h prior to study), refrain from strenuous physical activity for 48 h and alcohol for 24 h and avoid smoking on the morning of their visit.

Hyperinsulinaemic–euglycaemic clamp

A two-step hyperinsulinaemic–euglycaemic clamp with a stable glucose and glycerol isotope infusion was used to assess whole-body and tissue-specific insulin sensitivity. Upon arrival at the clinical research facility in King's College Hospital, participants were advised to empty their bladder and were weighed on a body composition analyser (Tanita MC780MA) to determine fat free mass and body weight for infusion calculations. A cannula for blood sampling was placed in the dorsum of one hand in a retrograde fashion, the hand was kept in a warming unit at 55° to mimic arterialised sampling. Duplicate baseline samples were taken to assess background glucose and glycerol isotopic enrichments. An infusion cannula was then inserted into an antecubital fossa vein on the adjacent arm for infusions of 20% (wt/vol) glucose, insulin (Actrapid, Novo Nordisk) bound to albumin in a 4% autologous blood/saline solution, [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol tracers (CK Gases, Cambridgeshire, UK). To begin the basal phase, a primed (2.0 mg/kg), continuous infusion (0.02 mg/kg^{-1} min^{-1}) of [6,6-$^2H_2$]-glucose and a primed (0.12 mg/kg), continuous infusion (0.0067 mg/kg^{-1} min^{-1}) of [$^2H_5$]-glycerol were initiated at −120 min (36). Blood samples were taken at −30, −20, −10 and 0 min for basal assessments. The clamp began at 0 min with a primed continuous insulin infusion at a rate of 10 mU m^{-2} BSA min^{-1} (low dose insulin phase) for 2 h for assessment of adipose tissue and hepatic insulin sensitivity. For the final 2 h, the [$^2H_5$]-glycerol isotope infusion was terminated, the insulin infusion rate was re-primed and increased to 40 mU m^{-2} BSA min^{-1} (the high dose insulin phase) for assessment of whole body and peripheral (skeletal muscle) insulin sensitivity (37, 38). Euglycaemia (5 mmol/L) was achieved using 20% glucose enriched with [6,6-$^2H_2$]-glucose (8 mg/g glucose with low-dose insulin and 10 mg/g with high-dose insulin) to maintain a constant tracer-to-tracee ratio. The glucose was given at variable rates, based on plasma glucose samples drawn every 5 min and measured on a bedside glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Yellow Springs, OH, USA). Blood was drawn at 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230 and 240 min for the assessment of plasma glucose concentration and isotopic enrichment and insulin concentration (38).

Magnetic resonance imaging and spectroscopy for ectopic fat quantification

MRI was used to assess visceral adipose tissue (VAT) and intrahepatic lipids (IHL). Proton-magnetic resonance spectroscopy (^{1}H-MRS) was used to assess intramyocellular lipids (IMCLs). Full details of this methodology can be found in (39). In brief, participants arrived at the MRI unit of Guy's Hospital, London following an overnight fast. Participants were scanned in a 1.5 T Siemens Aera scanner, axial two-point Dixon MRI images were acquired from the abdomen, from which fat and water images were produced. Images were analysed using imaging software (HOROS V 1.1.7; www.horosproject.org; accessed 21/10/2017) to quantify VAT and IHL. VAT area was assessed using a single slice at the L4-5 spinal anatomical position. IHL was quantified using two abdominal MRI images 30 mm apart encompassing both the superior and inferior view of the liver. A four-circular region of interest analysis was conducted to determine the hepatic fat fraction (%) in each region. IHL was calculated as the mean of all eight regions of interest. Quantification of IMCL in the soleus muscle of the right leg was derived from a ^{1}H-MRS scan on a 1.5 T Siemens system with an extremity RF coil to obtain muscle images. From these images two localised proton spectra were obtained, a water-suppressed lipid spectra and a lipid-suppressed water spectra. The Java-Based Magnetic Resonance User Interface software was used to identify and quantify the IMCL peaks expressed in arbitrary units (40).

Laboratory analysis

Plasma glucose and glycerol isotope enrichments were measured by gas chromatography-mass spectrometry on an Agilent GCMS 5975C MSD (Agilent Technologies) using selected ion monitoring to determine the tracer-to-tracee ratio. The isotopic enrichment of glucose was determined as the penta-O-trimethylsilyl-D-glucose-O-methoxime derivative (41). The isotopic enrichment of plasma glycerol was determined as the tert-butyl trimethylsilyl (tBDMS) glycerol derivative (42). Plasma insulin concentration was measured by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare).

Calculations

Whole-body insulin sensitivity was quantified using the M value (mg/kg FFM min^{-1}) measured during the final 30 min of the high-dose insulin phase of the clamp. It is calculated as total glucose disposal corrected for deviations in plasma glucose concentration. The M value was divided by mean plasma insulin concentration during the high dose insulin phase, giving M/I (mg kg FFM min^{-1})/(pmol/L) as another assessment of whole-body insulin sensitivity (37).

Steele's non-steady-state equations modified for stable isotopes (43) were used to determine peripheral glucose utilisation (glucose rate of disappearance, Rd (μmol/kg FFM min^{-1})), endogenous glucose production (glucose rate of appearance, Ra (μmol/kg FFM min^{-1})) and whole-body lipolysis (glycerol Ra (μmol/kg FFM min^{-1})) at basal and during the different phases of the clamp. Glucose kinetic calculations were modified to incorporate the [6,6-$^2H_2$]-glucose isotope enriched 20% glucose (44). Optical segment analysis was used to smooth the glucose and glycerol enrichment concentrations over the clamp time course (45).

Peripheral insulin sensitivity was determined as the percentage increase in the rate of glucose disappearance from basal to the final 30 min of the high-dose insulin phase. The peripheral insulin sensitivity index (PISI) was also calculated as the glucose Rd (μmol/kg FFM min^{-1})/ mean plasma insulin concentration (pmol/l) during the final 30 min of the high-dose insulin phase (46). Peripheral insulin sensitivity is predominantly driven by skeletal muscle glucose uptake but also captures adipose tissue glucose uptake, hence, we have used PISI as an assessment of skeletal muscle insulin sensitivity.

Endogenous glucose production (glucose Ra) was calculated by subtracting the exogenous glucose infusion rate from total glucose Ra. Hepatic insulin sensitivity was measured as the percentage suppression of endogenous glucose production from basal to the final 30 min of the low-dose insulin phase (47). Hepatic insulin sensitivity was also quantified during the basal and low-dose insulin phase using the hepatic insulin sensitivity index (HISI), which is the reciprocal of the product of endogenous glucose production (glucose Ra) and mean plasma insulin (46).

Adipose tissue insulin sensitivity was measured as the percentage suppression of whole body lipolysis (glycerol Ra) from basal to the final 30 min of the low-dose insulin phase (47). Adipose tissue insulin sensitivity was also quantified during the basal and low-dose insulin phase using the adipose tissue insulin sensitivity index (ATIS) which is the reciprocal of the product of whole body lipolysis (glycerol Ra) and basal plasma insulin (46).

Statistical analysis

The Soul–Deep II study was powered on a primary outcome of insulin secretory function (48). Allowing a difference of one S.D. to be detected with 90% power and two-sided significance, we aimed to recruit 23 per group, allowing for 20 per group to complete the protocol.

Data were assessed for normality using the Shapiro–Wilks test and histograms. A log 10 transformation was performed where data were skewed. Data which were normally distributed are presented as mean (S.D.), data which required log 10 transformation are presented as geometric mean (95% CI), data which remained skewed after log transformation are presented as median (lower-upper IQR). Ethnic comparisons of insulin sensitivity were assessed using the independent samples *t*-test for normally distributed data and Mann–Whitney test for non-normally distributed data. The mean difference (95% CI) and ratio of the geometric mean (95% CI) are presented where appropriate. Adjustment of the insulin sensitivity measures for VAT and IHL were made using linear multiple regression. Pearson's correlation analyses were used to assess the associations between insulin sensitivity measures and ectopic fat. Interaction by ethnicity was assessed using a linear multiple regression with ethnicity*ectopic fat depot used as the interaction term. Statistical significance was defined as $P<0.05$ and data analyses were performed using SPSS software, version 25 (IBM Analytics).

Results

Participant characteristics

The participant characteristics of the 21 BAM and 23 WEM are displayed in Table 1; by design the groups were comparable in age and BMI. There were no differences in body fat, waist circumference, blood pressure, HbA1c, cholesterol, fasting and post-load glucose between ethnicities; however, BAM exhibited lower fasting triglycerides compared to WEM. Data on ectopic fat depots in these volunteers, as previously reported by our group (39), showed lower VAT and IHL in BAM but similar IMCL (included in Table 1 for reference).

Whole-body insulin sensitivity

The glucose and insulin profiles during the hyperinsulinaemic–euglycaemic clamp are shown in Supplementary Fig. 1 (see section on supplementary materials given at the end of this article). BAM exhibited a trend towards greater mean plasma insulin during the high-dose insulin phase (ratio of the geometric mean and 95% CI of 1.10 (1.00, 1.21), $P=0.05$). There were no ethnic differences in whole-body insulin sensitivity, measured as either M value (BAM: 9.65 (2.32) vs WEM: 9.51 (3.86) mg/

Table 1 Participant characteristics. Data are presented as mean (S.D.) for normally distributed data.

Characteristics	BAM (n = 21)	WEM (n = 23)	P
Age (years)*	25 (22, 40)	29 (25, 53)	0.18
BMI (kg/m^2)	26.8 (3.6)	26.5 (4.5)	0.82
Waist circumference (cm)†	87.5 (83.4, 91.8)	92.8 (87.1, 99.0)	0.13
Systolic BP (mm/Hg)	124.0 (11.9)	121.9 (9.1)	0.52
Diastolic BP (mm/Hg)†	70.3 (65.5, 75.5)	70.7 (67.2, 74.3)	0.91
Total cholesterol (mmol/L)†	4.26 (3.85, 4.73)	4.65 (4.23, 5.11)	0.20
LDL (mmol/L)	2.73 (0.84)	2.99 (0.82)	0.33
HDL (mmol/L)*	1.2 (1.2, 1.4)	1.2 (1.1, 1.4)	0.86
Triglycerides (mmol/L)†	0.67 (0.59, 0.77)	0.99 (0.81, 1.21)	**<0.01**
Fasting glucose (mmol/L)	5.1 (0.5)	5.2 (0.4)	0.55
2-h post load glucose (mmol/L)	5.28 (1.13)	5.09 (1.26)	0.61
Ectopic fat depots			
Visceral adipose tissue (VAT), L4-5 (cm^2)†	46.1 (34.4, 61.7)‡	79.0 (55.4, 112.5)	**0.02**
Hepatic fat fraction (HFF) (%)	3.78 (1.13)‡	6.08 (5.04)	**0.04**
Intramyocellular lipid (IMCL) (AU)§	0.030 (0.015)	0.030 (0.014)	0.87

*data expressed as median (IQR) for non-normally distributed data; †geometric mean (95% CI) for log transformed data; ‡sample size=20; §sample size: BAM=18, WEM=22.

kg FFM min^{-1}, P=0.89) or M/I (BAM: 0.0171 (0.0059) vs WEM: 0.0189 (0.0094) ((mg/kg FFM min^{-1})/(pmol/L)), P=0.44). Associations between VAT, IHL and IMCL with whole-body insulin sensitivity (measured as either M value or M/I) are shown in Supplementary Table 1. When combining all participants as a single cohort, there were significant inverse associations between VAT, IHL, and IMCL with whole-body insulin sensitivity; however, when assessing the ethnic groups separately, these relationships were significant in WEM but weaker or absent in BAM.

Adipose tissue insulin sensitivity

Adipose tissue insulin sensitivity (ATIS) index did not differ by ethnicity during the basal or insulin-stimulated state (Table 2). Insulin-mediated suppression of glycerol was used as a measure of adipose tissue insulin sensitivity to lipolysis and was not significantly different by ethnicity (mean difference (95% CI) −8.55 (−22.0, 4.90)%, P=0.21), Supplementary Fig. 2A. There was a trend towards lower adipose tissue insulin sensitivity to lipolysis when adjusting for VAT in BAM (adjusted mean difference (95% CI) −12.4 (−26.9, 2.21)%, P=0.09). Across the whole cohort, adipose tissue insulin sensitivity did not correlate with VAT, IHL or IMCL (Fig. 1A, B and C). However, when assessing WEM and BAM separately, adipose tissue insulin sensitivity to lipolysis correlated with VAT and IHL in WEM, but there were no significant correlations in BAM (Fig. 1A and B). When modelled with suppression of lipolysis, ethnicity had no significant interaction with VAT (P=0.12) or IHL (P=0.58). There were no significant correlations between the suppression of lipolysis and IMCL in either ethnic group (Fig. 1C).

Peripheral insulin sensitivity

We found no ethnic differences in peripheral insulin sensitivity, measured as percentage increase in glucose utilisation from the basal to high-dose insulin phase of the clamp (BAM 304.82 (111.11) vs WEM 286.24 (138.44) %, P=0.63), Supplementary Fig. 2B. There was also no ethnic difference when accounting for the insulin concentration during the high-dose insulin phase, using PISI (mean difference (95% CI) -1.06×10^{-2} (-3.87×10^{-2}, 1.74×10^{-2}) (µmol/kg FFM min^{-1})/pmol/L, P=0.43), Table 2. Adjusting PISI for VAT (which we have previously reported as lower in BAM), resulted in significantly lower PISI in BAM (adjusted mean difference (95% CI) -3.47×10^{-2} (-5.67×10^{-2}, -1.27×10^{-2}) (µmol/kg FFM min^{-1})/pmol/L, $P<0.01$). Across the whole cohort, PISI correlated significantly with VAT and IMCL (Fig. 1D and E). When assessing WEM and BAM separately, PISI correlated significantly with VAT and IMCL in WEM; however, in BAM the association with VAT was weaker (Fig. 1D). and there was no association with IMCL (Fig. 1E). When modelled with PISI, interactions between ethnicity and VAT and between ethnicity and IMCL were not significant (P=0.11 and P=0.11, respectively).

Hepatic insulin sensitivity

We found no ethnic differences in the basal or insulin stimulated hepatic insulin sensitivity index (HISI), Table 2. Using suppression of endogenous glucose production as a measure of hepatic insulin sensitivity, we found no evidence for an ethnic difference (mean difference (95% CI) −4.15 (−14.83, 6.53)%, P=0.21), Supplementary

Table 2 Substrate kinetics before and after insulin adjustments during the basal state and the hyperinsulinaemic–euglycaemic clamp. Data are expressed as mean (S.D.)

	Basal state			Hyperinsulinaemic–euglycaemic clamp		
	BAM, $n=21$	WEM, $n=23$	P	BAM, $n=20$	WEM, $n=23$	P
Glycerol Ra (μmol/kg FFM min^{-1})	1.11 (0.71–2.72)*	1.55 (1.29–2.27)*	0.10	0.64 (0.52, 0.78)*	0.77 (0.63, 0.93)[¶§]	0.17
ATIS index (μmol/kg FFM min^{-1} × pmol/L)$^{-1}$	14.62×10^{-3} (10.36×10^{-3}, 20.70×10^{-3})†	12.16×10^{-3} (9.06×10^{-3}, 16.31×10^{-3})†	0.40	10.2×10^{-3} (4.61×10^{-3})	9.04×10^{-3} (4.46×10^{-3})	0.42
Glucose Ra (μmol/kg FFM min^{-1})	13.60 (1.24)	13.74 (1.33)	0.72	4.37 (3.94–5.64)*	3.23 (2.71–6.14)*‡	0.38
HISI (μmol/kg FFM min^{-1} × pmol/L)$^{-1}$	1.57×10^{-3} (6.61×10^{-4})	1.70×10^{-3} (7.18×10^{-4})	0.56	1.35×10^{-3} ($9.23–16.32\times10^{-4}$)*	1.68×10^{-3} ($11.57–23.59\times10^{-4}$)*‡	0.41
Glucose Rd (μmol/kg FFM min^{-1})	–	–	–	51.14 (44.61–60.16)*	50.48 (38.43–67.72)*	0.87
PISI (μmol/kg FFM min^{-1})/pmol/L	–	–	–	9.71×10^{-2} (3.43×10^{-2})	10.78×10^{-2} (5.32×10^{-2})	0.43

*data expressed as median (IQR) for non-normally distributed data; †geometric mean (95% CI) for log transformed data; ‡sample size:21; §sample size:22.
ATIS, adipose tissue insulin sensitivity; HISI, hepatic insulin sensitivity index; PISI, peripheral insulin sensitivity index; Ra, rate of appearance; Rd, rate of disappearance.

Fig. 2C. Adjusting hepatic insulin sensitivity for VAT, resulted in lower mean hepatic insulin sensitivity in BAM (mean difference (95% CI) −10.9 (−21.2, −0.72)%, P =0.04). Adjusting hepatic insulin sensitivity for IHL (which we have previously reported as lower in BAM), resulted in no ethnic difference in hepatic insulin sensitivity (adjusted mean difference (95% CI) −7.33 (−17.9, 3.24)%, P=0.17). Across the whole cohort, hepatic insulin sensitivity correlated with VAT and IHL (Fig. 1F and G). When assessing WEM and BAM separately, hepatic insulin sensitivity correlated significantly with VAT in both ethnicities (Fig. 1F); however, the correlation with IHL was only significant in WEM (Fig. 1G). When modelled with suppression of endogenous glucose production, interactions between ethnicity and VAT and between ethnicity and IHL were not significant (P=0.50 and P=0.66, respectively).

Discussion

In this study, we have shown that whilst BAM and WEM display comparable whole body, skeletal muscle, hepatic and adipose tissue insulin sensitivity, the relationships between insulin sensitivity and ectopic fat are ethnically distinct.

Resistance of adipose tissue to the antilipolytic effect of insulin is suggested to be a primary abnormality in the pathophysiology of T2D that occurs before the onset of hyperglycaemia (6). It has been proposed that VAT and ectopic fat accumulate as result of dysfunctional lipolysis, which allows an increase in circulating fatty acids, and other adipocyte abnormalities, described in the 'spillover theory' (10, 49). Our study, which is the first to compare men of black African and white European ethnicity, shows that there are no associations between lipolysis and VAT, IHL or IMCL in BAM. Our findings agree with Albu *et al.* who showed a relationship between suppression of lipolysis and VAT in obese white women but not in black women (31). Together, these findings suggest lipolysis may not be driving the accumulation of ectopic fat in black people of either gender, suggesting the 'spillover theory' may not hold true in this ethnic group.

Visceral fat, IHL and IMCL play an integral role in the development of insulin resistance and T2D (12, 16, 17); however, black populations are consistently reported to exhibit lower levels of VAT (19, 20, 21, 22, 23) despite their high T2D risk. Our finding of lower VAT in BAM is in agreement with the literature. Despite this, we found comparable levels of insulin sensitivity. We investigated

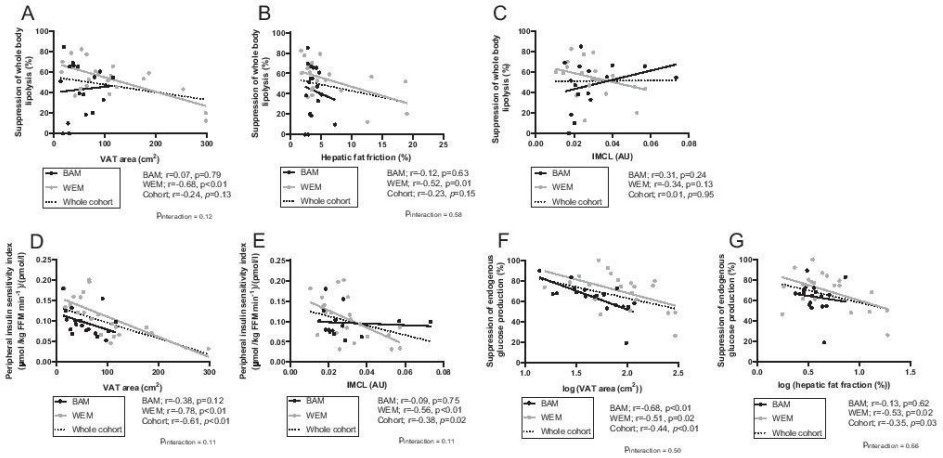

Figure 1

The association between the suppression of whole body lipolysis with VAT (A), hepatic fat fraction (B) and IMCL (C).The association between peripheral insulin sensitivity index with VAT (D) and IMCL (E). The association between the suppression of endogenous glucose production with VAT (F) and hepatic fat fraction (G). Data presented using the Pearson correlation coefficients. Peripheral insulin sensitivity index was measured during the high-dose insulin phase (40 mU m^{-2} BSA min^{-1}), suppression of endogenous glucose production and whole-body lipolysis was measured during the low-dose insulin phase (10 mU m^{-2} BSA min^{-1}).

European Journal of Endocrinology

associations between VAT and insulin sensitivity and found that both peripheral and hepatic insulin sensitivity were significantly associated with VAT in both ethnicities. This leads us to believe that VAT is detrimental to skeletal muscle and hepatic insulin sensitivity in both ethnic groups, but that this impact occurs at lower VAT levels in BAM compared to WEM, a so-called lower *threshold*. Our finding of a significant association between VAT and hepatic insulin sensitivity is consistent with earlier work in obese black women (25); however, our data in healthy men also show an association between VAT and skeletal muscle insulin sensitivity which has not been found in women (25, 50). This conflicting result may be due to the aforementioned studies focusing on women with severe obesity, whilst our participants were only mildly overweight or gender itself may explain the conflicting results, adding to the evidence for gender differences in T2D pathophysiology (33).

Accumulation of IHL is proposed to be central to the development of hepatic insulin resistance. The 'portal theory' describes the accumulation of IHL, which develops from the flux of fatty acids from VAT to the liver, via the portal vein. It is, therefore, not surprising that we found lower IHL in BAM, given the lower levels of VAT that they exhibited. Whilst our data from WEM corroborate the current understanding of T2D pathophysiology such that hepatic insulin sensitivity was significantly associated with IHL (14), we found no evidence for this relationship in BAM. This contrasts with data from studies in black women whereby IHL is associated with hepatic sensitivity (25, 26). This may point to IHL being more harmful in black women than men, although the obesity status of the women may also have contributed to this result (33).

Intramuscular lipids, which accumulate as a result of skeletal muscle cells taking up fatty acids from the peripheral circulation, have been shown to be correlated with skeletal muscle insulin resistance (15). Whilst we saw a significant relationship between IMCL and skeletal muscle insulin sensitivity in WEM, this relationship was not present in BAM. This finding agrees with other studies (51, 52, 53) and suggests that skeletal muscle insulin resistance develops independently of IMCL in BAM.

In contrast to the extensive evidence base that reports pronounced insulin resistance in populations of black African ethnicity (24, 54), we showed no ethnic differences in insulin sensitivity at a whole-body and tissue-specific level. The contrast in these findings are likely due to the different methodologies used to

measure insulin sensitivity. In our study we have used the clamp method, which is a direct assessment of insulin sensitivity (37), while other methods estimate insulin sensitivity through indirect modelling. The use of such methods in black populations has been criticised because of the reduced insulin clearance and higher insulin levels that they exhibit, which may lead to an underestimation of modelled insulin sensitivity. Indeed, in an ethnic comparison of direct and indirect measures of insulin sensitivity, Pisprasert *et al.* showed no difference in insulin sensitivity using the clamp, while surrogate indices showed greater insulin resistance in African-Americans compared to white Americans. These data suggest caution should be applied when using indirect assessments of insulin resistance in black populations (55). Our findings are supported by several metabolic studies using glucose clamps and isotopes, which have also found comparable insulin sensitivity in healthy black and white communities (25, 26, 55, 56, 57). Our experimental design also limited potential confounding factors; participants were similar in BMI, participants with impaired glucose tolerance were excluded and our data collection included only men.

We have previously published a description of the ectopic fat status for the current set of participants (39) in which we found no ethnic differences in IMCL, but significantly lower VAT and IHL in BAM. In the current study, we adjusted our insulin sensitivity data for VAT and found lower whole body, skeletal muscle and hepatic insulin sensitivity in BAM. The reduced insulin sensitivity following adjustment for VAT, and the lower VAT storage in the presence of similar lipolysis, suggests that the detrimental effects of VAT occur at lower levels in BAM and a resistance to storing VAT allows BAM to maintain comparable insulin sensitivity to WEM. In comparison, adjusting for IHL did not explain the similar hepatic insulin sensitivity and provides more evidence for an independent relationship between IHL and hepatic insulin sensitivity in BAM. Lower ectopic fat storage, despite similar lipolysis, may point to an increased tendency towards fat oxidation over ectopic storage in BAM; further studies assessing fatty acid oxidation are needed to explore this possibility.

Although one of the strengths of this study was the use of rigorous measurements of insulin sensitivity and ectopic fat, we recognise that our conclusions for the associations between insulin sensitivity and ectopic fat may be limited by our sample size. While our sample size is comparable to other studies of this nature, it does affect the statistical adjustment for VAT/IHL and interaction analysis. Our insulin sensitivity data are based on lean mass assessed by bioimpedance methodology; this uses calculations which are not ethnically sensitive and could potentially lead to underestimation of lean mass and thus overestimating insulin sensitivity in BAM (58). Finally, although the aim of our recruitment was to achieve comparable BMIs in our groups, this resulted in a tendency towards lower waist circumferences in BAM, which may have also contributed to differences in the metabolic characteristics that we studied. A study in which the groups are matched for waist circumference would help to elucidate these effects.

In summary, our data suggest that increased lipolysis due to adipose tissue insulin resistance may not be driving ectopic fat deposition in BAM. Additionally, ectopic fat accumulation in the liver and skeletal muscle may play less of a role in reducing insulin sensitivity in BAM compared to WEM. We provide evidence that the detrimental effects of VAT on glucose uptake and the suppression of endogenous glucose production occur at a lower VAT level in BAM. We conclude that current theories regarding the accumulation of ectopic fat and its impact on insulin sensitivity may not apply in BAM, who display a resistance to storing visceral and hepatic fat. Future work, assessing the impact of ectopic fat on insulin secretory function, is vital before excluding ectopic fat as the culprit behind the increased prevalence of T2D in black populations.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EJE-19-0636.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

L M G, S A A, A M U, J L P designed the study; O B, O H, M L, C M, F S-M, G C-E acquired the data, O B, O H, F S-M, A M U performed the data analysis, O B L M G, S A A, A M U contributed to the interpretation, O G, L M G drafted the article and all authors contributed to revising the intellectual content before approving the final article. J L P is supported by the NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London and is an NIHR Senior Investigator. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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